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HYDROGEN ION DETERMINATION[1]

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IN a half-hour discussion of hydrogen ions (hydrated hydronium ions) one may consider only a few points and many interesting facts are omitted. Since the dissociation of water gives rise to both hydrogen ions and hydroxyl ions, a water solution always contains both these ions, and since at a given temperature a change in hydrogen ion concentration is always accompanied by a reciprocal change in hydroxyl ion concentration, we do not ordinarily separate the effects of hydrogen ions from those of hydroxyl ions. At present we will consider aqueous solutions within a reasonable range of temperature. In the laboratory one may work at constant temperature, and in tropical seas the temperature is more or less constant around 29°.

It has been shown by physical chemists that the activity of hydrogen ions as affecting different properties of a solution may have different coefficients, and hence we will define activity as that affecting electrode potentials and indicators and particularly distinguished from conductance-ratios. The simplest method of determining hydrogen ion activity is by means of a pair of hydrogen electrodes (Fig. 1) connected through a salt bridge on one hand and on the other hand through a potentiometer. If

[1] Paper forming part of the symposium on "Hydrogen Ion Concentration" before the Ecological Society of America, meeting with the American Association for the Advancement of Science, Des Moines, Iowa, December 31, 1929.

the two solutions have the same hydrogen ion activity and temperature they will be at the same potential, and

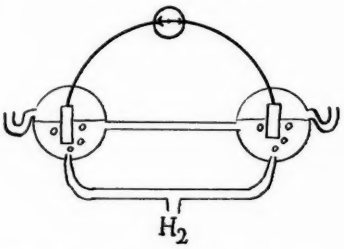

FIG. 1. Diagram of two hydrogen electrodes, the hydrogen bubbling through them, connected by a salt bridge (in the middle of the figure) and through a potential-measuring-device (in the upper part of the figure). This last in ordinary work is a potentiometer, but the details are not shown.

hence the hydrogen ion activity of an unknown solution may be determined by finding a solution of known hydrogen ion activity in the other electrode, which will be at the same potential. On the other hand, if one electrode contains ten times as much hydrogen ion activity as the other, there will be a difference of 59 millivolts in the potential of the two electrodes. This mathematical relation led Sörensen to put down his results on a logarithmic scale and denote by pH the logarithm of the reciprocal of the hydrogen ion activity, each unit change in pH causing a change in potential of 59 millivolts. Sörensen, however, standardized the solution in his reference electrode by means of conductance-ratios, and hence all his values of pH must be corrected by adding 0.04.

The practical use of the hydrogen electrode has met with some difficulties. In the first place, the surface of the electrode must be one which will absorb a store of hydrogen in the monomolecular form and quickly reach saturation. In order to prevent the loss of hydrogen due to its penetration into the interior of the electrode, electrodes have been made of gold, which absorbs very little hydrogen, and then electroplated with a metal which absorbs hydrogen more rapidly. Palladium may be used

for plating, but it is attacked by certain solutions. It may be removed with nitric acid and deposited as at first. A platinum surface has to be laid down under certain conditions which are empirically determined, but it may become poisoned so that it reaches equilibrium slowly in oxidizing solutions, and it can not be removed with nitric acid. About the only way to treat such an electrode is to replate it on top of the old poisoned surface. Iridium seems to be an excellent metal for the surface of the electrode. Whether the surface is applied smooth and bright or rough and black affects the determinations in various ways, particularly in the presence of oxidizing solutions. The electrode-surface acts as a catalyst, causing a union of hydrogen with the oxidizing substance, which thus prevents a saturation of the electrode with hydrogen. If this catalytic action is very rapid, however, and hydrogen is supplied in gaseous form, it should be possible to reduce the whole solution by means of the electrode until saturation with hydrogen is possible. A particular difficulty in biochemical measurements arises from the fact that the passage of CO_2 into the hydrogen may change the hydrogen ion concentration in the solution. In the Clark electrode the hydrogen may be retained while part of the solution is changed, in this way bringing new samples of the solution in relation to the hydrogen which has come to equilibrium with the earlier samples. If the solution is viscous, as blood plasma, the McClendon electrode vessel may be used for shaking the hydrogen with some of the solution in one compartment, then with a new portion of the solution in the second compartment containing the electrode. For solutions which are not viscous the electrode must be completely immersed when taking the reading. If the solution contains oxygen the electrode must be shaken until all the oxygen is reduced by means of the hydrogen. It is therefore unwise to use rubber stoppers in electrode vessels or rubber connections for hydrogen gas since both oxygen and CO_2 may diffuse through the rubber. The hydrogen from a cylin-

der may be passed over heated platinized asbestos to remove the oxygen. If the two hydrogen electrodes, one containing a standard solution of hydrogen ions, are at different hydrogen ion activities, there will be a diffusion potential in the salt bridge connecting the electrodes. This potential may be reduced by making the salt bridge of a saturated solution of KCl, since the potassium ions travel at about the same rate as the chlorine ions, and most of the current is carried by potassium and chlorine ions. If, however, the hydrogen ion activity in one of the electrodes has a very high value, there will be an appreciable amount of the current carried by hydrogen ions which will give rise to a higher diffusion potential. The same would be true of hydroxyl ions in very alkaline solutions. In most biochemical work, however, the solutions are often near neutrality, and hence a salt bridge of saturated KCl may reduce the diffusion potential to a low value. Diffusion potentials not only cause error in the determination but they may vary from moment to moment and give an inconstant reading. This is due to a change in the character of the junction brought about by diffusion. In order to set up junctions that are constant, various forms of flowing junctions have been devised in which the current opposes the effect of diffusion on the distribution of ions. Although the liquid junction potential may be reduced by using a saturated KCl solution, it is not absolutely eliminated, and furthermore, it is not constant but changes with time. Therefore, it seems a waste of time to attempt to get hydrogen electrode readings in smaller units of potential than a millivolt. In order to maintain constancy of the standard electrode, it should be filled with a buffer solution, which is essentially a mixture of an equivalent number of molecules of a weak acid and its salt with a strong base. A calomel electrode may be substituted, which is essentially pure mercury covered with a layer of pure calomel in a KCl solution. The concentration of the KCl affects the solubility of the calomel and hence the potential of the

electrode. In the 0.1 N KCl calomel electrode the electric conductivity of the KCl solution is so low as to reduce the accuracy of the readings, hence the normal KCl calomel electrode or the saturated KCl calomel electrode has been substituted. In the last named the calomel is mixed with crystals of KCl. Since the solubility of calomel is affected by temperature, and time is required for equilibrium, there is a time lag in all calomel electrodes after a change of temperature. This lag is greatest in the saturated KCl calomel electrode, since the temperature affects the solubility of KCl as well as of calomel. This is just another reason for working at constant temperature. Whether a calomel electrode or a standard hydrogen electrode is used at constant temperature, the pH is a linear function of the potential difference and addition is all that is required in constructing a table for converting millivolts into pH. In routine determinations, if it is necessary to have flowing junctions or other elaborate arrangements, the time required for each determination may be very great. For this reason other methods have been used for determining hydrogen ion activities, and the hydrogen electrode has been reserved for standardization. Of the other electrode methods, the quinhydrone electrode is perhaps the most reliable. The quinhydrone electrode consists of a platinum wire dipping in an unknown solution, which is saturated with quinhydrone. This is connected by a salt bridge to a calomel electrode. Often the platinum wire is moistened and dipped into the quinhydrone and stirred in the unknown solution. Sometimes the quinhydrone tends to float on top as a dry powder and is difficult to wet. The liquid junction potential vitiates this method as it does the hydrogen electrode method. One is here measuring an oxidation-reduction potential which is influenced by the hydrogen ion concentration. Quinhydrone is a combination of one molecule of quinone and one of hydroquinone and on dissociation gives rise to the same concentration of quinone and hydroquinone, and to this extent the oxidation-reduc-

tion potential is constant and the hydrogen ion activity the only variable. If, however, the solution contains substances which will oxidize hydroquinone or reduce quinone appreciably before the potential readings can be made, the method can not be used. The sources of error in the method are the liquid junction, impurity of the quinhydrone, failure to saturate the solution with quinhydrone, loss of CO_2 and failure to regulate the temperature. The quinhydrone electrode was standardized by means of the hydrogen electrode, and it seems probable that each laboratory should standardize it independently. Conversion tables can be made in the same way as for the hydrogen electrode. After standardizing at two H ion activities (since the pH is a linear function of the potential), it is not even necessary to know the formula. Escape of CO_2 will change the hydrogen ion activity in the quinhydrone electrode in the same way as it will in the hydrogen electrode, but since it is not necessary to have a gas-phase, this escape may be easily prevented if some arrangement for stirring in the quinhydrone without opening the apparatus is provided. Rubber stoppers and connections here also would be undesirable because of their permeability to CO_2, but as a rule the reading can be taken very quickly, and perhaps the best approach to accuracy is made by working rapidly.

In case a solution is not very turbid, its hydrogen ions may usually be determined by indicators. The change in color of an indicator is associated with a tautomeric change in the molecule with the migration of the double bond, perhaps involving a quinoid form. But this tautomeric change depends on the ionization of the indicator or undissociated-salt-formation. The majority of indicators widely used are weak acids (or behave as such, dissociating hydrogen ions) and obey the law of mass action so that their dissociation depends on the hydrogen ion activity according to the equation, $\frac{(H^+) \times (A^-)}{(HA)} = K$,

or if the dissociation is represented by α, the equation becomes $\frac{\alpha(H^+)}{1-\alpha} = K$, in which (H^+) denotes H ion activity. At 50 per cent. dissociation of the indicator $\alpha = 1 - \alpha$ and $(H^+) = K$. Therefore $\log \frac{1}{(H^+)} = \log \frac{1}{K}$. Hence, in order to choose a series of indicators covering a pH range, we arrange them in increasing order of value of $\log \frac{1}{K}$. Clark and Lubs have given us a series of indicators arranged in this way which is very useful. The above equations are for monovalent acids, and in case of polyvalent acids the values of K for the different dissociations must be very far apart, else the dissociation curves will overlap. Clark and Lubs have used sulphonic acid dyes in which the dissociation of the sulphonic acid group is very great compared to the other dissociations in the dyes. Hence the sulphonic acid dissociation does not overlap the other dissociation curves. Since K varies with temperature, it is necessary here to work at a constant temperature just as with electrodes. It was shown by McClendon and Magoon that the hydrogen ions in bicarbonate solutions or blood plasma do not appreciably change with change in temperature between 20° and 40°. But the hydroxyl ions are greatly influenced by temperature. Furthermore, the colors of indicators in the blood plasma are influenced by temperature.

Neutral salts affect the dissociation of indicators without change of pH due to the effect of the ionic strength on the activity coefficients of the indicator, and probably do not change the dissociation constant. But the salt effect may be expressed in terms of change in apparent dissociation constant.

Protein affects the color of the indicator. The effect of protein is minimized by dilution, but dilution also changes the hydrogen ions. Since the indicator method must be standardized by the hydrogen electrode, the standardization eliminates the error of dilution by using

the undiluted solutions with the hydrogen electrode and the diluted with the indicator. Blood plasma should be diluted between twelve and twenty times with CO_2-free water. The dilution must be the same in the standardization as in the other determinations.

It seems to me that the enormous growth of biochemistry has been somewhat associated with the use of the Duboscq colorimeter, and this is an admirable instrument with which to determine hydrogen ions by means of indicators. In case the undissociated indicator is colorless and its ions colored, the depth of color is directly proportional to α and may be determined directly by means of the Duboscq colorimeter using as a standard a solution of the same concentration of the same indicator in which dissociation is made practically complete (by adding alkali). If the thickness of the layer of the unknown is made 1 cm, for instance, the thickness of the layer of the standard in centimeters when a color match is obtained $= \alpha$, since the concentration of the color is inversely proportional to the thickness. If $\log \frac{\alpha}{1-\alpha}$ is measured on the ordinate and pH on the abscissa the curve of dissociation of the indicator will be a straight line, since $\text{pH} = \log \frac{1}{K} + \log \frac{\alpha}{1-\alpha}$. The midpoint of the curve is determined by the circumstance as previously stated, that when $\alpha = \frac{1}{2}$, $\text{pH} = \log \frac{1}{K}$. If the units on abscissa and ordinate are equal, each dissociation curve will form an angle of 45° with the axes. The value of $\log \frac{1}{K}$ is marked on the x axis (abscissa) and a straight line at 45° drawn through it. In this way a dissociation curve for the second indicator may be determined. The accompanying chart (Fig. 2) shows such curves for a number of indicators. Values of α are shown on the right.

In constructing the chart values of K taken from Kolthoff were usually chosen in case several values of K were

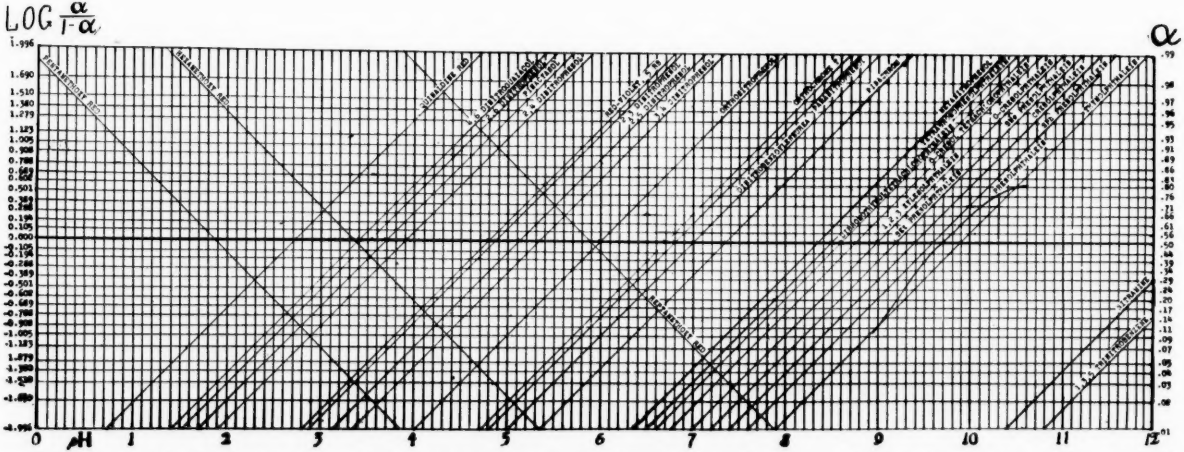

FIG. 2. Graph of a series of one-color-indicators, showing the dissociation and apparent dissociation constants. On the ordinate is measured $\log \frac{\alpha}{1-\alpha}$ and on the abscissa the pH, but on the x axis (abscissa) (where $\log \frac{\alpha}{1-\alpha} = 0$), the values of $\log \frac{1}{K}$ (or the logarithm of the reciprocal of the dissociation constant) are measured for each indicator, and the graphs are straight lines running through these points. Indicators are not entirely divided into acid and basic. Three basic indicators that are colorless in alkali and colored in acid show a slope from below upward toward the left. The indicators whose graphs are in the reverse directions are colorless in acid and colored in alkali, irrespective of whether they are acid or basic, but most of them are acids. Phenolphthalein has three dissociation constants close together, and the graph is made from the data of Michaelis. On the right the values of α are marked for convenience in determining hydrogen ion concentration.

found in the literature. Each laboratory should standardize the method by means of the hydrogen electrode at the temperature and salt concentration desired. The advantage of the chart is that the dissociation curve of an indicator under given conditions of temperature, salt and protein concentration need be standardized at only one point and a straight line drawn through that point making an angle of 45° with the x axis.

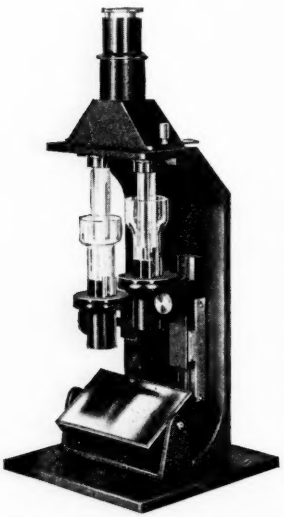

FIG. 3. Dubosecq Colorimeter.

This method is very convenient for determining the pH of a solution, such as blood plasma, which must be enclosed to prevent the escape of CO_2. In the type of Dubosecq colorimeter used (Bausch and Lomb biological model), a glass cup of unit depth when closed by a glass cover is provided. This cup is filled with the solution of indicator in CO_2-free water. Blood plasma is drawn up into the very fine pipette and the end of the pipette inserted to the bottom of the cup. One tenth cc of the blood plasma is allowed to run out into the cup where it forms a layer under the indicator solution. The cover is slipped in place, thus preventing any diffusion with the air, and the cup is rotated, thus mixing its contents. The cup is placed on one side of the colorimeter. On the other the standard indicator solution (completely dissociated) may be exposed to air, hence the plunger may be used to vary its depth.

These little cups of unit depth, when closed with a cover, are very convenient for compensating for the color of the unknown solution. Some of the unknown solution without the indicator may be placed on the same side of

the colorimeter which contains the standard solution of indicator.

Another method of eliminating the error due to color of the unknown solution is by the use of monochromatic light in determining α. Many indicators show an absorption band spectrum. Monochromatic light of wavelength near the middle of this absorption band is used. It is very probable that it will be in a region of the spectrum in which there is little absorption due to the color of the unknown solution. This method is useful also in some cases where the undissociated indicator is slightly colored.

Sulfonphthalein indicators of Clark and Lubs may be used in the Duboscq colorimeter if the light passes through a solution of the indicator in its yellow state. This solution may be put over the eye-piece or between the source of light and the colorimeter. Although this is not monochromatic light, it is a light in which the interfering region of the spectrum is filtered out.

For obtaining approximately monochromatic light usually at least two (superimposed) color filters are necessary. A convenient source of light is rarefied gas made luminous by passing an electric current, thus showing a line spectrum. The mercury arc, as well as neon tubes and tubes of other gases, which are so popular now in electric signs, are very useful as sources of light. With monochromatic light and the Duboscq colorimeter, one is provided with a spectrophotometer of very high accuracy and rapidity of action at a very low cost. The problem then resolves itself into one of hunting for proper indicators and color filters. The Wratten filters are very useful but not absolutely permanent, and a number of glass filters have been made by the Corning Glass Works.

THE pH REQUIREMENTS OF CULTIVATED PLANTS IN NATURAL AND ARTIFICIAL CULTURES[1]

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THE factors which are operative and cause cultivated plants to vary in their pH requirements, when grown in either natural or artificial cultures, are as yet not very clearly understood, although it has been known for a long time that certain species of plants are affected unfavorably by soil acidity or what is sometimes called a lack of lime, while in this respect other species are indifferent or may even be affected favorably. The relation of soil reaction to plant distribution has been discussed extensively in recent years by Arrhenius (1922), Christophersen (1925), Olsen (1921), Wherry (1927) and others. On the basis of deportment towards lime, Contejean (1881) about fifty years ago made a botanical classification of plants under three general heads: *viz.*, the calciphile, or lime-loving, the calcifuge, or lime-avoiding, and the indifferent. While this system of classification may be valuable from a botanical standpoint, it is not adapted for use in a detailed consideration of the relation of soil acidity and lime to our common agricultural plants, for with a few exceptions all these plants grow best on soils well supplied with lime. This point is further emphasized by Hilgard (1905), who has frequently called attention to the adage, "A limestone country is a rich country," and also the statement of Hall (1908) that any soil containing less than 1 per cent. of calcium carbonate will be benefited by liming. The beneficial effects of

[1] Paper forming part of the symposium on "Hydrogen Ion Concentration" before the Ecological Society of America, meeting with the American Association for the Advancement of Science, Des Moines, Iowa, December 31, 1929.

liming soils on agricultural plants are often due partly to indirect actions of the lime, in which case the use of the term calciphile, or lime-loving, might carry the wrong impression as to the mode of action of the lime.

In this connection it is interesting to note that experiments by Pipal (1916), of the Indiana Station, and White (1916), of the Pennsylvania Station, show that sorrel (*Rumex acetosella*), which is commonly supposed to be a lime-avoiding plant, is really benefited by liming. As they, and also Frear (1915) and others, have indicated, sorrel is usually found growing on very acid soils because there it meets with the least competition from other weeds and also field crops. It would be found growing even better on limed soils were it not that here other plants grow so well and are so vigorous that they crowd out the sorrel. Undoubtedly, competition working in conjunction with soil acidity or alkalinity in the way just indicated has a marked influence on the powers of different species of plants for establishing and maintaining themselves under natural conditions (Hall, 1910), and thus has affected the character of the native vegetation found in many regions.

The pH Relation is Complex

The subject of the relation of soil reaction to the growth of plants in soil cultures is really so complex that a great deal of confusion has arisen as to just how reaction influences growth. In artificial cultures it is possible to control the conditions fairly well, so that only the direct influence of reaction limits growth. The indirect and direct influences of reaction on plant growth in soil cultures may be summarized as follows:

A. The indirect influence of reaction on plants due to effects of reaction on factors as follows:
   1. Physical condition of the soil.
   2. Availability of essential elements.
   3. Activity of soil micro-organisms.
   4. Solubility and potency of toxic agents.

5. Prevalence of plant diseases.
6. Competitive powers of different species of plants.

B. The direct influence of reaction on plants
1. In extreme acidity or alkalinity, may have a toxic or destructive effect of excess of H or OH ions on plant tissues.
2. May have unfavorable balance between acidic and basic constituents available for absorption by plants.

In properly controlled artificial cultures, it should be possible to limit the influence of reaction to the direct one.

THE INDIRECT INFLUENCE OF REACTION ON PLANTS

The physical condition of clay soils, especially, is generally known to be affected unfavorably by an acid condition, because of an insufficient supply of calcium bicarbonate in the soil solution to keep the clay particles flocculated, which condition is necessary for the highly desirable granular or crumb structure. In sandy soils the presence of lime carbonate probably improves the physical condition by acting as a binding agent. In improving the physical condition of soils in these ways, the presence of lime carbonate thus indirectly helps in the proper regulation of the air and moisture supply of soils, which, in turn, is the fundamental basis for developing the proper biological and chemical activity of soils. Both a highly acid condition or alkaline condition may, by inducing deflocculation, cause a movement of colloidal material from the surface soil into the subsoil where it may be precipitated in the form of a hardpan. The depletion of the surface soil and the formation of the hardpan usually have an unfavorable influence on fertility.

The availability of all the essential elements secured from the soil is affected one way or another by the reaction. Phosphorus, in particular, becomes less available with increasing acidity. Recent investigations by Ford and the writer show conclusively that this is due, in at least many cases, to the interaction of soil phosphates with hydrated iron oxides to form a basic iron phosphate which has a very low availability. The presence of an

abundance of lime prevents this interaction by keeping the phosphate tied up with calcium in the form of the easily available calcium phosphate. Our results indicate that a pH of 6 or higher is required to keep the phosphorus in readily available form. Yellow soils, often poorly drained, owe their color to hydrated iron oxide, and if they are acid, the phosphorus is usually of low availability and the soils are rather infertile. This condition may have a marked effect on the native vegetation on account of variations in the ability of different species of plants to feed on difficultly available phosphates.

The influence of reaction on the availability of potassium is not very clearly understood as yet. With increasing acidity, calcium and magnesium become less available, because of a decreasing supply and a greater competition for them by the relatively insoluble soil acids. This may markedly affect plant growth. Iron and manganese appear to become less available with decreasing acidity, at least up to a certain point, and their availability may become a limiting factor in plant growth in certain cases.

The activity of soil micro-organisms is greatly influenced by reaction, and this in turn affects the availability of the nitrogen, sulphur and other elements whose liberation is dependent on the decomposition of organic matter. Fred and Davenport (1918) and Bryan (1923b) have shown that many species of the nitrogen-fixing bacteria are especially sensitive to acidity and may exist only for a comparatively short period in distinctly acid soils. The relation of soil micro-organisms to the pH requirements of plants in soil cultures is a very important one.

Increasing acidity favors the solubility and potency of toxic compounds of aluminum, copper and zinc, and soluble aluminum is held by some (Abbott, 1913; Burgess and Pember, 1923; Hartwell and Pember, 1918; Mirasol, 1920) to be a very important factor in the relation of reaction to plant growth. Magistad (1925), found that

at a pH lower than 5 and higher than 9, aluminum may become sufficiently soluble to be seriously toxic to plants. Copper, zinc and other heavy metals are usually not present in sufficient amounts in soils to be toxic even at high acidities.

Certain fungous diseases like "finger-and-toe" develop to a harmful extent only in acid soils, while other plant diseases like the potato scab are most serious in recently limed soils, and hence at least slight acidity is desirable under many conditions for potato growing. Aside from the cases known to have a direct relation to plant diseases, there are a few special plants like the cranberry, blackberry and water-melon for which a soil of at least slight acidity seems desirable and in some cases necessary for the best growth. It is not known just why these plants grow better on an acid soil, but it seems possible that in some cases plant diseases or malnutrition, due to a lack of iron where lime is added, may again be factors.

The influence of soil acidity or alkalinity on the competitive powers of different species of plants for establishing themselves and crowding out others has already been discussed and its method of operation explained. Under native conditions or in case land is left in meadow or pasture for a considerable number of years, this influence may be a factor of considerable importance. However, under most farming conditions, which are the primary concern of this paper, this form of competition is largely eliminated by cultivation, and hence need not receive further consideration in this discussion.

THE DIRECT INFLUENCE OF REACTION ON PLANTS

The work of several investigators (Hoagland, 1919; Truog, 1918) indicates that a direct toxic or destructive effect of an excess of H or OH ions on root tissues probably does not take place except at extreme acidity and alkalinity. The reaction of the root sap of most agricul-

tural plants falls in the pH range of 4 to 6, indicating that plant tissues are so constituted as to stand the acidities ordinarily found in soils. Extreme OH-ion concentrations, such as are represented by a pH greater than 9, probably have a direct toxic effect in many cases.

The balance between basic and acidic constituents available for absorption by plants varies directly with the reaction and is probably the most important factor involved in the relation of reaction to plant growth. Before discussing this relation, it seems desirable to discuss the cause and nature of soil acidity, briefly.

The Origin and Nature of Soil Acidity

During the past year, Mr. Chucka and the writer have determined positively that the insoluble inorganic acid causing soil acidity is an alumino-silicic acid of the empirical formula $Al_2O_3 \cdot 4\,SiO_2 \cdot X\,H_2O$. It is a white substance, insoluble and quite resistant to attack by alkalis and acids. It exists usually in colloidal form and like many zeolites has the property of readily exchanging one base for another or for hydrogen. A suspension of the acid has a pH of approximately 3. When partly neutralized it is an excellent buffer. In the form of the sodium salt or as the free acid, it disperses to a high degree when electrolytes are absent, causing a running of soils. When the acid is saturated with calcium, it flocculates completely and gives a soil the desirable granular structure. It has an enormous water-holding capacity and, in short, exercises a controlling influence on the physical and chemical properties of soils.

The substance has been isolated from soils and also the rock called bentonite. It has been prepared in the writer's laboratory by treating albite with a 1 per cent. solution of sodium bicarbonate, which has a pH of 8.4, and also by simply boiling powdered albite with water. Albite and other feldspars in contact with water hydrolyze and impart to the water a pH of approximately 8.4.

At this pH the feldspars are unstable and one third of the silica splits off, leaving the alumino-silicic acid. Under natural soil conditions, between periods of rain and leaching, the films of water surrounding the particles of feldspar attain a pH of about 8.4 and then the acid is formed. Since approximately 60 per cent. of the solid crust of the earth is composed of feldspars, and water is the most abundant natural reagent, the wide and universal distribution of the compound is readily explained.

As soils weather and leach, the bases become depleted and there is left an excess of this alumino-silicic acid only partially saturated with bases. The soils thus become acid. The nitric and sulphuric acids which are formed in the decomposition of organic matter can become neutralized only partially, on account of difficulty of securing bases from the partially saturated alumino-silicic acid or base exchange compound. As a result, the soil solution becomes acid, and we have an acid mineral soil of the common type.

Our investigations indicate that there exists in soils at least one organic acid, probably included in the humic acid group, which is insoluble and has properties nearly identical with those of the alumino-silicic acid just described. Peats and black prairie soils may owe their acidity quite largely to this organic acid, while mineral soils low in organic matter owe their acidity largely to the alumino-silicic acid.

It should be clearly recognized that a soil is not a homogeneous medium and that even in distinctly acid soils there are particles of feldspar and other minerals whose water films may be distinctly alkaline at times. Plants may thus feed in localized alkaline areas, even in acid soils.

THE BALANCE BETWEEN BASIC AND ACIDIC CONSTITUENTS REQUIRED BY PLANTS

It is obvious that if plants were dependent entirely on the soil solution for their needs of essential elements

from the soil, and if the bases and acids were absorbed in the proportion existing, there would be absorbed an excess of acids over bases in the case of acid soils. This sort of a situation might be more favorable for one species of plant than another, depending on the proportion of acids to bases required by the plant in its metabolism. An examination of the composition of different species of plants as regards their basic and acidic constituents should throw some light on this matter. In Tables I and II these data for alfalfa and timothy are presented.

TABLE I

ALFALFA—BASIC AND ACID CONSTITUENTS IN WATER-FREE MATERIAL*

Basic constituents			Acidic constituents		
Constituent	Per cent.	Oxygen equivalent lbs. in 100 lbs.	Constituent	Per cent.	Oxygen equivalent lbs. in 100 lbs.
Al_2O_3	.054	.0253	SiO_2	.070	.0187
Fe_2O_3	.020	.0060	Cl	.100	.0225
CaO	2.970	.8460	P_2O_5	.638	.2157
MgO	.578	.2286	SO_3	.527	.1053
K_2O	2.146	.3640	N	2.674	1.5280
Na_2O	.208	.0537			
MnO	.004	.0009			
Total		1.5245	Total		1.8902
			Less 2/3 N taken from air		1.0186
			Total taken from soil		.8716

Ratio is – 1.75 of bases to 1 of acids.

* Data based on analyses by Magistad of five Wisconsin field samples cut in hay stage.

These data show that alfalfa requires almost twice as much bases as acids and that as a consequence nearly one half of the bases must enter in the form of the carbonate or bicarbonate. A base entering as the carbonate or bicarbonate is for metabolic purposes a free base, because of the weakness of carbonic acid. In contrast

TABLE II

TIMOTHY—BASIC AND ACIDIC CONSTITUENTS IN WATER-FREE MATERIAL*

Basic constituents			Acidic constituents		
Constituent	Per cent.	Oxygen equivalent lbs. in 100 lbs.	Constituent	Per cent.	Oxygen equivalent lbs. in 100 lbs.
Al_2O_3	.053	.0249	SiO_2	1.851	.4910
F_2O_3	.155	.0465	Cl	.583	.1315
CaO	.332	.0947	P_2O_5	.474	.1602
MgO	.220	.0871	SO_3	.460	.0920
K_2O	2.190	.3688	N	.990	.5657
Na_2O	.017	.0043			
MnO	.006	.0013			
Total		.6276	Total		1.4404

Ratio is – .44 of bases to 1 of acids.

* Data based on analyses of three field samples cut in bloom period. See U. S. D. A. Bur. Soils, Bul. 600, 1917, p. 11, for all analyses except nitrogen, which is taken from Henry and Morrison, "Feeds and Feeding."

to this, the timothy plant requires approximately twice as much acids as bases and hence none of the bases need enter in the form of the carbonate or bicarbonate. Alfalfa is known to be much more sensitive to acidity than timothy, and these data offer an explanation of this, based on the metabolic requirements of the plants. Generally speaking, agricultural crop plants grow best in the pH range 6 to 8.

An explanation is also offered why alfalfa and other legumes dependent on symbiotic nitrogen fixation are affected more by acidity than when they draw their nitrogen from the soil. When nitrogen is taken from the soil as nitrate, it brings along with it a certain amount of base. When it is taken from the air, no bases come with it. It is commonly assumed that legumes may secure two thirds of their nitrogen from the air, and hence in the calculation of the alfalfa data this amount has been subtracted from the acidic constituents.

Silica constitutes about one third of the acidic constituents of timothy, and it might be argued that this

should be omitted because it is a non-essential. Granting this, the acidic constituents are still greatly in excess of the basic.

On the basis of this explanation, the capacity of plants to feed locally on particles of limestone, feldspar, the base exchange compounds and other minerals and, in some cases, in the deeper, less acid and even calcareous subsoils, determines to some extent the ability of the plants to grow in acid soils. How plants may feed locally, independently of the soil solution, has been discussed by the writer (Truog, 1927) and referred to as solid phase feeding. By solid phase feeding is not meant that solid particles are absorbed by plants, but that through plant intervention, solid material is brought into solution and subsequently absorbed. Solid phase feeding may be beautifully illustrated with an experiment as follows.

Experiment Illustrating Solid Phase Feeding

Make an artificial root hair out of collodion, using a small test-tube for the forming vessel. It is essential that a test-tube be selected which has an absolutely smooth interior surface; otherwise the sack is liable to leak. Fill the sack with carbonated water, using a funnel, and then with a moistened camel's-hair brush, paint the outside of the sack with powdered calcite or precipitated calcium carbonate. At the end of thirty minutes, or better, an hour, remove the carbonated water with a pipette and test it for the presence of calcium by adding a little ammonium oxalate and boiling for a few minutes. If the experiment has been properly carried out, a slight but distinct test for calcium will be obtained, showing that solution in the form of the bicarbonate and absorption have taken place.

Because of the delicacy of the phosphate test, a more striking demonstration can be made in a short time if finely ground rock phosphate is used in place of calcium

carbonate. At the end of thirty minutes sufficient phosphate will have dissolved and entered the sack to give a pronounced test for phosphate. To test for phosphate, remove five cubic centimeters of the solution from the sack, place in a small vial and add four drops of the ammonium molybdate-sulphuric acid reagent prepared as directed by Truog and Meyer (1929). Now add a crystal of stannous chloride about one half the size of a pin-head, or a drop of stannous chloride solution, and shake. A distinct blue color will be produced, showing that solution and absorption of phosphate have taken place.

This experiment illustrates how plants growing in a soil with an acid soil solution may, by so-called solid phase feeding, absorb an excess of bases over acids and thus meet their requirements. Bases absorbed in the form of the carbonate or bicarbonate serve the same purpose as free bases because of the weakness of carbonic acid. The comparative ability of plants in meeting their specific requirements of bases is dependent on solid phase feeding power, the rate of growth of the plant and the amount of bases required. These matters have already been discussed in detail elsewhere (Truog, 1918).

THE pH REQUIREMENTS OF PLANTS IN ARTIFICIAL CULTURES

In artificial cultures, such as water solution and sand cultures, plants are dependent for their mineral elements entirely on what may be absorbed from solution. It is thus difficult in studies of this kind to make direct comparisons between natural soil cultures and artificial cultures. Artificial cultures are, however, better adapted for the study of the influence of pH on plant growth because the feeding on the solid phase can be eliminated.

The greatest problem with artificial cultures is the one of controlling and maintaining a definite pH. In an attempt to accomplish this, investigators have either used

large vessels or have made frequent changes of the solution. Various buffers have also been used, but their effectiveness is limited on account of the choice and amount permissible. The writer suggests the possibility of using the base exchange compound, that is, the alumino-silicic acid causing soil acidity, as a solid buffer for sand cultures. This would create a condition more nearly like that in soils and make it possible to control the pH to a high degree. The fulfilment of this possibility is dependent on the preparation of the base exchange compound in quantity and in pure form. The writer sees no reason why this can not be done.

A large number of tests (Bryan, 1922, 1923a, 1923c) have shown that in artificial cultures, alfalfa, medium red clover, alsike clover, soybeans and in fact most legumes grow best in the pH range 6 to 8. Cereals and many non-legumes grow best over a slightly more acid range.

Summary

The reaction of the soil influences plant growth both directly and indirectly. The indirect influence results from the effect of reaction on the physical condition of the soil, the availability of the essential elements, the activity of soil micro-organisms, the solubility and potency of toxic agents, the prevalence of plant diseases and the competitive powers of different species of plants.

A direct toxic or destructive effect on the plant tissues probably takes place only in the most extreme cases of acidity and alkalinity. More often the direct detrimental effect is probably due to an unfavorable balance between acidic and basic constituents available for supplying the metabolic requirements of the plant. The data relating to the composition of plants support this view; *e.g.*, alfalfa, a sensitive plant to acidity, requires approximately a two to one ratio of bases to acids, while timothy, which is much less sensitive, requires, similarly, approximately a one to two ratio.

The soil is not a homogeneous medium as regards reaction, and plants may adjust their requirements of bases by feeding more strongly on the less acid portions of the soil.

Generally speaking, under natural soil conditions, agricultural crop plants grow best in the pH range of 6 to 8. Probably because of indirect effects, a few agricultural plants grow better at a more acid reaction than pH 6.

In artificial cultures most legumes grow best in the pH range 6 to 8, while cereals and many other non-legumes may grow best over a range slightly farther to the acid side.

LITERATURE CITED

Abbott, J. B., Conner, S. D., and Smalley, H. R.
1913. "Soil Acidity, Nitrification and the Toxicity of Soluble Salts of Aluminum," Ind. Agr. Exp. Sta. Bul. 170.

Arrhenius, O.
1922. "Hydrogen-ion Concentration, Soil Properties and Growth of Higher Plants," *Arkiv För Botanik*, 18: 1–54.

Bryan, O. C.
1922. "Effect of Different Reactions on the Growth and Nodule Formation of Soybeans," *Soil Sci.*, 13: 271–302.
1923a. "Effect of Reaction on Growth, Nodule Formation and Calcium Content of Alfalfa, Alsike Clover and Red Clover," *Soil Sci.*, 15: 23–35.
1923b. "Effect of Acid Soils on Nodule-forming Bacteria," *Soil Sci.*, 15: 37–40.
1923c. "The Effect of Different Reactions on the Growth and Calcium Content of Oats and Wheat," *Soil Sci.*, 15: 375–381.

Burgess, P. S., and Pember, F. R.
1923. "'Active' Aluminum as a Factor Detrimental to Crop Production in Many Acid Soils," R. I. Agr. Exp. Sta. Bul. 194.

Christophersen, Erling.
1925. "Soil Reaction and Plant Distribution in the Sylene National Park, Norway," *Transactions* of the Conn. Academy of Arts and Sciences, 27: 471–577.

Contejean.
1881. "Géographie botanique. Influence du terrain sur la végétation," 143 pp., Paris. (From Hilgard, E. W. [1905], p. 521.)

Frear, W.
1915. "Sour Soils and Liming," Pa. Dept. Agr. Bul. 261, p. 101.

Fred, E. B., and Davenport, Audrey
1918. "Influence of Reaction on Nitrogen-assimilating Bacteria," *Jour. Agr. Res.*, 14: 317–336.

Hall, A. D.
1908. "Fertilizers and Manures," p. 253. E. P. Dutton Company, New York.
1910. "The Adaptation of the Plant to the Soil," *Jour. Roy. Hort. Soc.* (London), 36: 1–21.
Hilgard, E. W.
1905. "Soils," p. 365. Macmillan Company, New York.
Hartwell, B. L., and Pember, F. R.
1918. "Unlike Effect of Acid Soils on Plants Due to Aluminum," *Soil Sci.*, 6: 258.
Hoagland, D. R.
1919. "Relation of the Concentration and Reaction of the Nutrient Medium to the Growth and Absorption of the Plant," *Jour. Agr. Res.*, 18: 73–117.
Magistad, O. C.
1925. "The Aluminum Content of the Soil Solution and its Relation to Soil Reaction and Plant Growth," *Soil Sci.*, 20: 181–225.
Mirasol, J. J.
1920. "Aluminum as a Factor in Soil Acidity," *Soil Sci.*, 10: 153–218.
Olsen, C.
1921. "The Concentration of Hydrogen-ions in the Soil," *Science*, n. s., 54: 539–541.
Pipal, F. J.
1916. "Red Sorrel and Its Control," Ind. Agr. Exp. Sta. Bul. 197.
Truog, E.
1918. "Soil Acidity: I. Its Relation to the Growth of Plants," *Soil Sci.*, 3: 169–195.
1927. "How Plants Feed," *Proceedings and Papers*, First International Congress of Soil Science, Washington, D. C., 3: 628–636.
————, and Meyer, A. H.
1929. "Improvements in the Denigès Colorimetric Method for Phosphorus and Arsenic," *Indus. and Engr. Chem.*, Anal. Edition, 1: 136–146.
Wherry, E. T.
1927. "Divergent Soil Reaction Preferences of Related Plants," *Ecology*, 8: 197–206. Gives references to numerous other papers by Wherry.
White, J. W.
1916. "Concerning the Growth and Composition of Clover and Sorrel as Influenced by Varied Amounts of Limestone," Pa. Agr. Exp. Sta. Ann. Rpt., 1913–14, pp. 46–67.

THE RELATION OF pH TO PLANT DISTRIBUTION IN NATURE[1]

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INTRODUCTION

FOURTEEN years have elapsed since Dr. E. T. Wherry (1916) first applied the modern method of measuring soil acidity towards the solution of plant distribution problems. Since this classical work Wherry and many others have produced hydrogen-ion papers in almost colossal proportions. Mevius (1927) writes a comprehensive review of soil reaction as it affects both cultivated and wild plants and gives 759 titles. Arrhenius (1926) in his review uses 403 titles. The author, however, will use and cite only such works as seem pertinent for the purpose of this paper. Although the attack has been concentrated on the higher plants, the lower, green ones, at least, do not remain unscathed, so that we have soil acidity studies from algae to orchids. By virtue of his sustained and vigorous activity Wherry is still the leading pH investigator among plant ecologists in America. Ironically enough, if we except his work, soil acidity investigations though begun in America have attained their fullest expression in Europe. Some of the European workers, as we shall soon see, have been singularly precise in their determinations and thought provoking in their carefully prepared presentations.

The once apparently narrow and novel subject of soil reaction has by this time developed into a very broad field. Without necessarily ignoring the perfectly patent influence of soil acidity on plant distribution, most investigators soon realized that other factors were opera-

[1] Paper forming part of the symposium on "Hydrogen Ion Concentration" before the Ecological Society of America, meeting with the American Association for the Advancement of Science, Des Moines, Iowa, December 31, 1929.

tive. More and more species found outside of their so-called natural ranges have quite necessarily generated many points of view regarding the whole subject of soil acidity. Some still consider pH *the* factor; others, *a* factor; and there are those who consider its influence unimportant in their particular studied species. Some consider its influence direct; others, indirect. Some say it is hydrogen-ion *per se*. Also, some writers lay much stress on attending factors. The latter view complicates the matter uncomfortably, for it forces us to recognize an abstruse triangular interrelationship among hydrogen-ion concentration, plants and attending factors, or what Clements calls concomitants. In the following pages we can only suggest the present status of pH work. In doing so we might first make a preliminary survey of the whole plant kingdom by beginning with the higher plants and ending with the algae.

Members of Plant Kingdom Investigated

Seed plants.—Following Coville's (1913) lead, Wherry has shown that the ericads are, with few exceptions, notably acid-loving species. The reader is referred to his summary of 1920 for his thought and work to that date. Only a few species are found to reach over into alkaline soils. Wherry (1920) has also shown a very restricted range for the orchids, nearly all of them being confined to decidedly acid habitats. If the reader consults the work of Arrhenius (1920), Olsen (1921), Salisbury (1921), Atkins (1922), Chodat (1924), Kelley (1923) and Christophersen (1925), he will find many other seed plants with high correlation between pH and distribution. On the other hand, it must be brought out that trees, as a group, are notably indifferent. See Kotilainen (1927), Geisler (1926), Salisbury (1921) and Kurz (1923). Miss Geisler also points out that a number of the herbaceous flowering plants of the forests tolerate a wide range.

Ferns.—Wherry (1920), Steagall (1926) and Robinove and La Rue (1928) have given special consideration to

the ferns and present their findings in convenient tables. The results of all three investigators indicate that most species of ferns for which the data are at all comprehensive tolerate a wide range of acidity. Wherry, however, assigns optimum reactions to nearly all his species. Most of Robinove and La Rue's species show a wide range of tolerance extending from definitely acid to alkaline habitats. Only a few, where the number of tests was limited, proved the exception, as Robinove and La Rue expected. But the data of Steagall and Wherry extended even their range considerably: consult Table I, which is an adaptation from Robinove and La Rue, Wherry and Steagall.

Table 1

	Species	No of tests	pH range	Optimum reaction
Robinove and La Rue	*Cystopteris bulbifera*	3	7.4-8.2	7.0
Wherry	" "	30	5.5-8.5	7.0
Steagall	" "	12	6.0-8.0	7.0
R and L R	*Aspidium spinulosum*	8	4.8-6.4	
W	*var intermedium*	20	4.5-8.0	
S	" "	10	5.5-7.0	6.0
R and L.R	*Pteris aquilina*	3	4.6-6.8	
W	" "	30	4.5-6.5	5.5
S.	" "	6	5.5-6.0	5.5
W.	(Southern) *Camptosorus rhizophyllus*	50	5.5-8.5	7.0
S.	" "	10	4.5-6.5	5.5
W.	" *Asplenium platyneuron*	50	4.5-8.5	4.5
S	" " "	13	5.0-6.5	6.0
W.	(Northern) *Polypodium vulgare*	50	5.0-8.5	6.0
S.	" " "	12	4.5-5.0	5.0
W.	(Southern) *Polypodium polypodioides*	15	4.5-7.0	4.5
S.	" " "	10	4.5-7.0	6.5
W	" *Pellaea atropurpurea*	30	5.5-8.5	7.0
S	" " "	15	7.5-8.0	8.0

In the second part of the table the writer advisedly pits a part of Wherry's data against Steagall's. As expected, Wherry's individuals, because of the greater number of tests, show in the main wider ranges than Steagall's. Both investigators assign an optimum reaction to their species. In accord with Wherry's practice, the reaction most frequently encountered is called the optimum reaction. The optimum thus roughly conforms with the frequency mode of the statisticians. Wherry sees a relationship between "soil reaction and geographic range." The writer can do no better than quote him in part from pages 262–264 (1920):

It is noteworthy, however, that the peculiar relations found to exist among rock ferns—the favoring of acid soils by southern species and of circumneutral soils by northern ones—is likewise well marked in the present series of plants. As the same sort of relation appears also with other plants than the ferns, in particular with the native orchids, it is sufficiently definite to justify inquiry into its probable origin. . . .

The territory left bare by the retreat of the great ice-sheet must at first have presented an almost unbroken expanse of circumneutral soils, and the vegetation which first occupied it accordingly comprised only plants which thrive best in such soils. Although acid soils have developed subsequently in many places, and permitted invasion by plants adapted to growth under acid conditions, a considerable number of the original occupants still persist, and are to-day classed as "northern" species.

In more southern regions, on the other hand, decomposition usually outstrips disintegration, so that soils containing undecomposed carbonate minerals are relatively rare. Except where limestone outcrops or where leaf mold accumulates, therefore, the dominant soil reactions are inclined to be acid, and the plants, established there since long before the glacial period, have become adapted to growth in such soils. The favoring of circumneutral soils by northern species, and of acid soils by southern ones, is thus connected with the geological history of the respective regions.

If we refer to the table we will notice that the optima of Wherry and Steagall do not always coincide. In making a meteorological study of southern Illinois, Miss Steagall finds that the latter differs very little from Virginia (southern limit of Wherry's fern study) except that the summer temperature in July may average 20° F. higher than in Virginia. According to Miss Steagall, this extremely high July temperature, together with the low rainfall—sometimes less than one half inch in July—rather than soil reaction, accounts for the distribution of rock ferns in southern Illinois. Here, too, we shall rely on quotations from pages 134–135, Miss Steagall (1926):

Polypodium polypodioides, a southern fern, in Wherry's tests run into maximum specific acidity 300 [pH 4.5]; *Polypodium vulgare* found its maximum at 10 [pH 6.0]. In southern Illinois tests *P. polypodioides* had its maximum at specific acidity 3 [pH 6.5], although it showed itself capable of living in higher acidities; *P. vulgare* had its maximum at 100 [pH 5.0], and did not run lower anywhere. What is the factor that reverses these fern habitats between Virginia and southern Illinois? As has been shown, the striking difference between the Virginia and the southern Illinois climate is the intense heat of summer. Since *P. polypodioides* is better adapted by its structure to endure these conditions, it runs ten to

fifty feet higher on the sandstone, where there is little soil but its own frond mold. As a consequence its soil tests run the gamut of acidities from one [pH 7.0] to 300 [pH 4.5], only with the difference that here its maximum runs at acidity 3 [pH 6.5]. *P. vulgare,* on the contrary, less xerophytic in structure, adheres to the lower rocks where there is more soil, even when this soil be weathered to acidity 100 [pH 5.0].

Camptosorus rhizophyllus in a cooler climate grows on calcareous rock, and there finds the right amount of moisture when specific acidity is one, but in southern Illinois that it may get sufficient moisture it must resort to sandstone with maximum 30 [pH 5.5].

The distinguishing factor between north and south, as far as the plant is concerned, is not the presence or absence of calcareous rock, as has been suggested. These are abundant in both regions. Temperature has changed, and this factor affects the amount of soil water and the amount of transpiration. This factor then has driven the semi-xerophytic forms from the dry limestone to a medium where moisture is available.

Even while we hold this judgment in abeyance we can not keep from seeing that Dr. Wherry has opened up a highly suggestive train of thought which Miss Steagall has met in a highly interesting manner.

Mosses and liverworts.—The almost universal correlation between Sphagnums and acid substrata is well known. But Robinove and La Rue (1928), already quoted, have recently given us some data for thirty-two species of liverworts and sixty-two of true mosses. Of the ninety-four species, thirty-eight were tested in less than five situations each, and only twelve species in ten or more habitats. They worked in a region having chiefly sandy soils so that naturally most habitats are of acid reactions. In such a region one would not expect many species or individuals in alkaline substrata; yet 34 per cent. show a wide range, twelve of the thirty-two of the so-called indifferents growing in soils all the way from definitely acid to those having a pH of 7.0 or more. The indications are that mosses and liverworts are not sensitive to reactions; however, they realize that their data do not warrant any general conclusions concerning the reaction preference of Bryophytes.

Algae.—Wehrle (1927) has given us a fascinating account regarding the reaction and distribution of algae. His studies comprise many species found in brooks, pools,

ponds and moors. He classifies his algal habitats into four categories as to hydrogen-ion concentration, each type being characterized by its own algal flora. In the highly acid waters of the moors the reaction is relatively constant, being regulated by humus acid buffers. In such waters the species are few but the number of individuals enormous. Strongly alkaline waters are also few in species and rich in individuals. The latter waters are also relatively constant as to reaction because of the buffer action of the lime content. By far the greatest number of species were found in weakly acid waters (pH 5.0–7.0). Most of these species tolerate a wide range. Some of his alkaline waters are rich in lime and some almost lime free. Yet 85 per cent. of the species of the former habitat are also found in the latter. He concludes, therefore, that the pH must be the factor.

If the waters are not strongly buffered he finds pronounced vacillation in pH. Much of the acidity of such habitats is due to carbon dioxide. In one pool containing *Nitella* in the upper strata of water the pH would rise (acidity increase) as the day progressed. *Nitella* carrying on photosynthesis consumed the carbon dioxide accumulated from the respiration of water organisms. In the lower strata the pH also increases but after noon decreases again. Presumably the respiration of mud organisms liberates more carbon dioxide than is consumed in the lower strata where light radiation is weaker, except at midday (see Fig. 1). If the water was quiet he found pH 7.9 at the surface where the light was strongest; about 25 cm deeper he obtained pH 6.5, and at the bottom where the light was weakest, pH 6.0. This rather nice stratification was often obliterated by convection currents set up by jumping frogs as the investigator approached the pool. We have here a beautiful example of the nicety of balance in nature.

Wehrle gives the ranges for over three hundred species. Some species show very restricted and others very wide ranges, seemingly independent of pH. Interest-

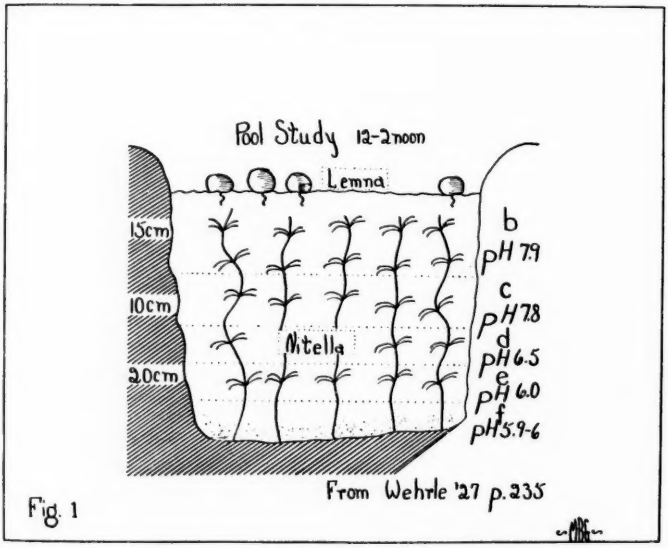
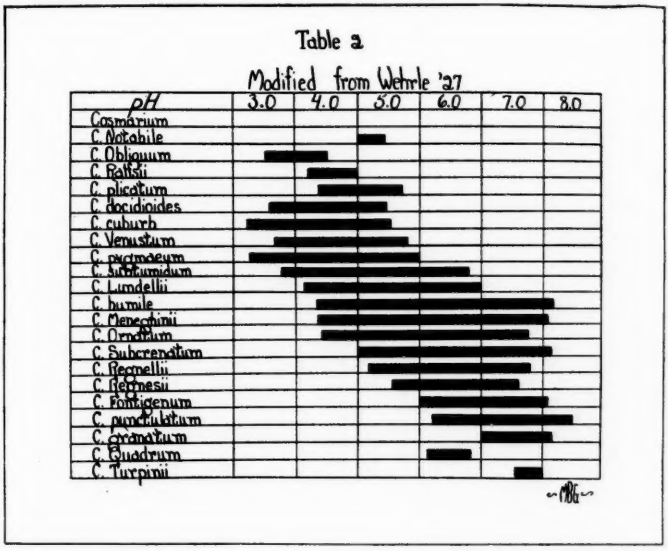


Fig. 1

ingly enough we find all degrees of tolerance in a genus if only it have a large enough number of species. The writer has tabulated just enough of his *Cosmariums* to show the enormous variation of tolerance among species of the same genus (refer to Table II).

Ulehla (1923) illustrates the importance of reactions on the growth of algae in a neat experiment. It seems that one of the Oedogoniums (*O. psychohormium*) has specialized cells called psychohormia. These cells are incrusted with calcium salts, usually calcium carbonate. Ulehla submerged this *Oedogonium* in water acidulated with carbonic acid. If subjected to sunlight for thiry minutes the reactions of the water changed from pH 6.90 to pH 7.05 because the photosynthetic action of the alga used the carbon dioxide introduced into the water. He repeated the experiment but placed the culture in darkness for thirty minutes. The reaction again changed from pH 5.85 to pH 6.18. This time, Ulehla explains, the calcium carbonate incrustations on the alga neutral-



ized the reaction of the water to the point most favorable for the alga. If kept in darkness for a day or two the alga died, because after all the calcium was exhausted the reaction of the water became acid by the plant's liberation of carbon dioxide during its respiration. This *Oedogonium,* presumably a calciphyte, dies in acid reactions. He often finds this and other calciphytic algae on shell animals where the buffer reaction of the calcareous shells keeps the water locally neutralized.

In nature he finds that species of *Cladophora* and *Oedogonium* are covered with the iron bacteria *Sideromonas confervarum* whose iron and calcium carbonate incrustations work as a buffer. As carbon dioxide accumulates in the water by respiration calcium bicarbonate is formed. This compound liberates hydroxyl-ions which neutralize the hydrogen-ions. Without the iron bacteria the *Cladophoras* and *Oedogoniums* referred to occur in alkaline waters attached to limestone and sandstone which furnish local buffering.

Lichens.—Trümpener (1926), dealing with species of tree lichens, finds that most of them are grouped around certain pH values (see Table III). He considers pH an

Table 3

Table I. Frequency of Lichens in pH-Intervals of the Substratum.

pH	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0
Xanthoria parietina							2	2	1	7	8	2	4	5	4	1	9	1	1	3
Physica grisea								1	1	2	2		2	1		1	1			
Physica pulverulenta							1	2	1	10	1	1	2	2		1	3			
Anaptychia ciliaris			2		1		1	1		1	1		1	1		2				
Parmelia acetabulum			1	1	3	2		2	1	1	2		2	2	1	2				
Xanth. candalaria					1	2	1	5	1	3	1		1	1	1					
Lecanora varia			4	5	6	3	2	3	3	3	4	3	5	4						
Physcia ascendens			1		1		3	3	2	4	2	1	2	1						
Physcia tenella				1	2		3	3	1	7	3		1							
Ramalina pollinaria				1	1		2	2		2	2	1								
Ramalina fraxinea			1		1	1	2	2	1	3	1									
Ramalina farinacea		2		1	1		2	1	1	1	1									
Parmelia subaurifera		1	2	2	1	2	4	1	1	2										
Parmelia sulcata		1	2	1	2	1	3	1	1	1										
Parmelia saxatilis		1	1	1	3	1	8	1	2	1										
Parmelia physodes		1	7	1	7	1	6	1	1	1										
Pertusaria communis	2	1	1	1	1	1	2	1	1											
Evernia prunastri	3	4	7	1	1	1	6	1												
Lecanora Subfusca						1	1	3	2	3	3	3	4	3	5	4				

Trümpener '26

essential factor in the distribution of lichens. These reactions vary for tree species as well as for species of lichens. But at the same time he points out that the cause for various affinities between nitrophilous species and tree species is not the pH of the tree bark, but rather the various ammonium contents of the differently constituted humus which the lichens receive or "catch."

The gist of his work, if the reader interprets him correctly, is that there are nitrophobous and nitrophilous tree lichens. The nitrophobes betake themselves to the top where by necessity they must grow on a more highly acid substratum. The nitrophiles, on the other hand, betake themselves to the bases where their nitrogen requirement is present. After the nitrogen requirement is met the various species group themselves around characteristic pH values.

Some Special pH Correlations

pH, leaching and successions.—From the foregoing survey of the plant kingdom and pH it will be seen that a good inroad has been made into all major divisions. We might now consider in particular a few special cases of broader pH interrelationships, for example, pH to plant succession and leaching. Salisbury (1921) has shown that leaching and removal of carbonates result in accumulation of humus and proportional increase in acidity. He gives examples of oak forests invading hill-tops as humus and acidity develop. Humus favors moisture conditions of the soil and therefore germination of forest tree species. In some cases, however, leaching has resulted in soils so acid that germination and growth of tree species become impossible. Many of the typical moors with their heath and *Pteris* vegetation owe their initiation to such leaching and concomitant extreme acidity. On the gentle slopes of hills where leaching sometimes lags will be found such herbs as *Mercuriales perennis* or shrubs like the English yew on alkaline soil. If

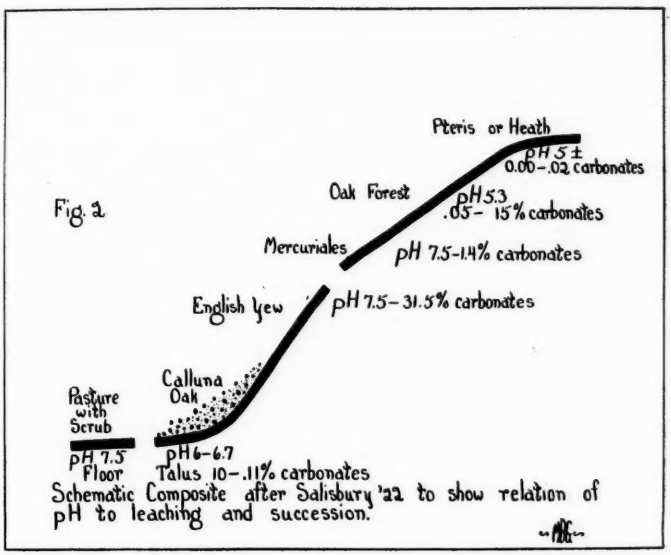
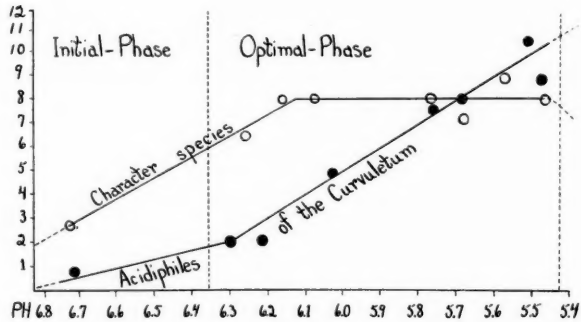


Fig. 2. Schematic Composite after Salisbury '22 to show relation of pH to leaching and succession.

the slope is steep enough, leached soils may be removed and deposited near the bottom as acid talus. In such cases there will also be an acid vegetation at the base of the slope, while over the valley floor alkaline shrubs and grasses usually prevail. In general he sees forests advancing downward from the higher elevations as a result of progressive downward leaching. In extreme cases more acid moors and vegetation in turn chase the once dominating and acid oaks from the top table lands (see Fig. 2). Braun-Blanquet and Jenny (1926) have shown how indifferent associations first inhabit circumneutral soils; how such an association slowly gives way to a more acidic one, and how the latter is finally replaced permanently by the curvuletum, an acidic climax (see Fig. 3). They have also shown how leaching and accumula-



Curve of the character species and direction of succession in the Elynetum(Elynetum[moderately acid]→Curvuletum[very acid])
Fig. 3 from Fig. 19 Braun-Blanquet & Jenny '26

tion of humus parallel these successional states. In other words, simultaneously with the development of an acidic climax vegetation there is also a development of an acidic climax, humus soil.

pH and soil profiles.—In Fig. 4 Braun-Blanquet and Jenny present also soil profiles to show four stages of humus soil development in the Alps. A condensed explanation follows. The pioneer stage consists of only a

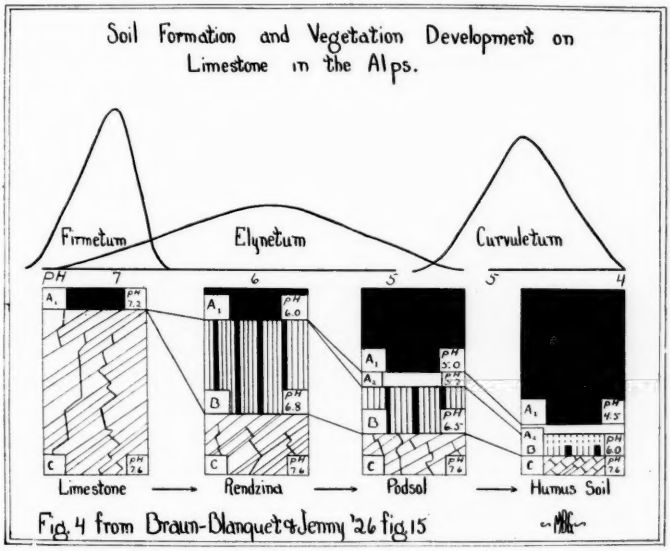


Fig. 4 from Braun-Blanquet & Jenny '26 fig. 15

humus layer (the A-horizon) and an unchanged calcareous mother rock (the C-horizon). At this stage the *Firmetum* (*Carex firma*-association) takes possession. This association consists of alkaline or circumneutral reaction species. As humus accumulates and carbonates are leached out there results a clay or loam horizon (B)—a layer rich in minerals derived from the partially depleted A-horizon. Now the *Elynetum* (*Elyna myosuroides*-association), because of its relative indifference to great pH variability, replaces the *Firmetum*. Luxuriant plant growth increases the humus and acidity of the A-horizon. As a consequence, leaching increases so that the latter horizon becomes completely depleted of its carbonates. Horizon A attains now an acidity of pH 5.0. Next the upper part of the B-horizon becomes

leached of bases and bleached in color. This pale layer indicates the beginning of the degeneration of the B-horizon. There are now four horizons: (1) a relatively thick and well-leached and acid humus A_1-horizon; (2) a white or gray leached A_2-horizon differentiated from the upper part of the B-horizon; (3) a diminished B-horizon possessing among others iron and aluminum compounds leached from above, and (4) the mother rock C. Such a soil profile is usually referred to as a "podsol," a term of Russian origin. Ordinarily the podsol is considered the climax soil, but Braun-Blanquet and Jenny consider the podsols as transitory in the higher Alps where they are superseded by the climax humus soils. These humus climax soils are distinguished by a higher acidity, a thicker humus A_1-horizon and the very diminished and indiscernible A_2- and B-horizons. This humus climax soil is inhabited permanently by the *Curvuletum* (*Carex curvula*-association), an acid climax vegetation which has its incipiency during the previous podsol stage at which time the *Elynetum* is replaced.

Negative evidence between pH and succession.—Quite in contrast Miss Geisler (1926), an American worker, saw no relations between pH and plant successions, but found on the contrary that her climax habitats in the vicinity of Cincinnati exhibited a wider range of acidity than the pioneer calcareous soils. The writer (1923), too, has reported that the sandy soils of certain beech-maple-hemlock forests run almost the whole gamut of reactions. On page 20 we read,

> The sandy flood-plain has a very irregular H-ion concentration, varying from 1 to 300 [pH 7.0–4.5]. Alluvial material washed in from the upstream moraine probably is one of the factors operating toward neutrality. Decaying logs, organic matter, together with a high water table, on the other hand, increase acidity locally.

These are very patchy, unstable and in a measure exceptional conditions. Nevertheless, there it is, the climax forest with its typical climax forest undergrowth.

Plants as pH indicators.—Establishing the frequency with which certain species group themselves around given pH values, Olsen (1921) has been able to predict the acidity by taking the census of the species in a given association (consult Table IV). Here we find that the

Table 4

Meadow locality in Stroggaards Vang (arid forest)

Species	No. of Fr.	PH classes									
		35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79
Festuca rubra	100									
Holcus lanatus	100									
Anthoxanthum odoratum	100									
Geum rivale	100									
Plantago lancolata	100									
Rumex acetosa	60									
Galium palustre	40									
Poa pratensis	20									
Lathyrus pratensis	20									
Taraxacum sp.	20									
				1	4	8	8	5	4	2

Determination of pH by Vegetation
Instance 2 p. 61 Olsen '21
Both pH 5.5–5.9 & 6.0–6.4 probable ∴ calc. = pH 6.0
Soil sample = pH 5.8

values pH 5.5–5.9 and pH 6.0–6.4 contain the same number of species. Olsen therefore predicted the intermediate pH 6.0. The soil really tested pH 5.8 so that he came remarkably close.

Kotilainen (1927) makes graphs (Figs. 5 and 6) to express the percentage of times that certain species or associations participate in given reactions. Observing then the typical species of the habitat and applying these graphs he is able to predict reactions. From his graphs it will be seen that the plant association, because of its greater restriction to narrower pH limits, is more accurate than a single species. Since pH indicates a set of conditions he is able to determine the agricultural value of his moors by the vegetation. For example, *Meny-*

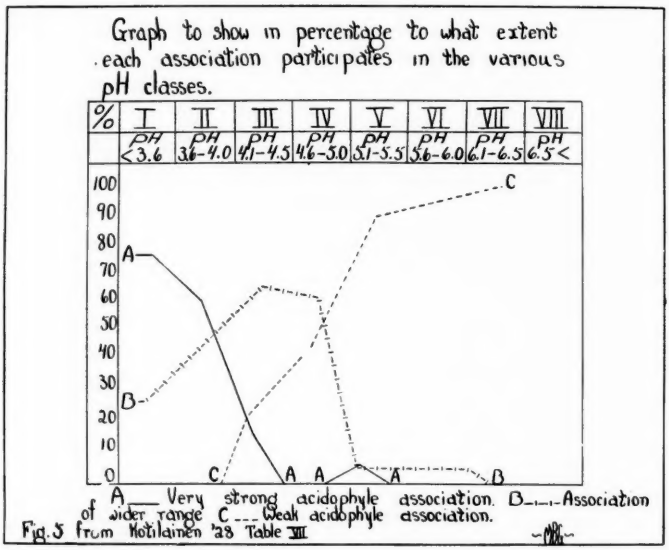


Fig. 5 from Kotilainen '28 Table III

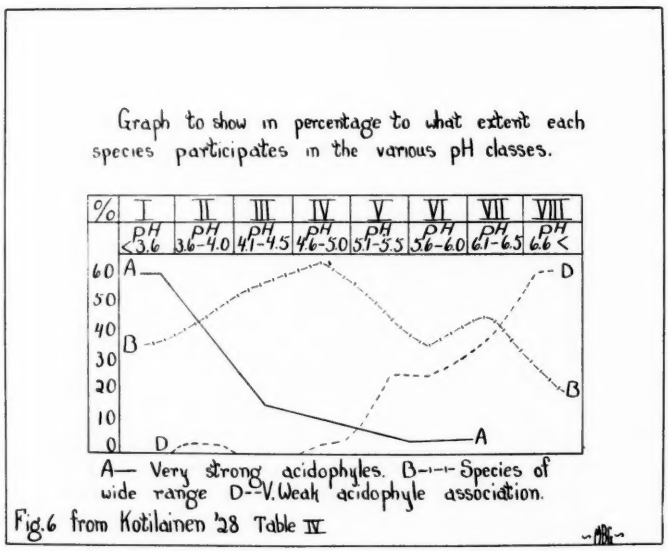
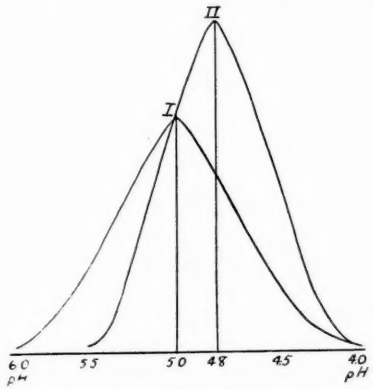


Fig. 6 from Kotilainen '28 Table IV

anthes trifoliata, Phragmites communis, Cypripedium calceolus and *Juniperus communis* indicate good peat for culture, but *Ledum palustre, Calluna vulgaris* and *Empetrum nigrum* indicate great acidity and poor peat for culture.

Frequency curves.—Braun-Blanquet and Jenny (1926) made a frequency study of pH of the *Carex curvula*-association and of *Carex curvula* individually. From the values they derived two typical bell-shaped curves. The dispersion is narrower and the mode higher for the



pH-variation curves of the species Carex curvula (I) and the association Curvuletum (II) (for a 100 individuals each)
Ordinate: frequency
Abscissa: pH

Figure 7 from Figure 5
Braun-Blanquet and Jenny '26 R.S.

association than for the species. These two curves also demonstrate that the association is more reliable as an indicator than the species. Applying Gauss's formula which is familiar to all statisticians and substituting normal distribution values they predict the probability of finding a given species in the habitat of a given pH (consult Table V).

Mono- and bimodal curves.—Salisbury (1925) gives a number of bimodal and monomodal distributional curves. For illustration see Fig. 8 with *Mercuriales perennis* and its bimodal curve. As if to disarm possible critics he points out that the frequency of the soil's pH and plant

Table 5.

Frequency table of the pH values of Carex curvula (125 Samples)

pH values (x)	6.0	5.9	5.8	5.7	5.6	5.5	5.4	5.3	5.2	5.1	5.0	4.9	4.8	4.7	4.6	4.5	4.4	4.3	4.2	4.1	4.0
Frequencies observed (y)	0	1	2	1	3	4	8	10	10	13	14	15	11	12	8	7	3	0	2	1	0
Frequencies calculated (y)	0.2	0.5	1.0	1.9	3.3	5.1	7.4	9.8	12.0	13.5	14.1	13.5	12.0	9.8	7.4	5.1	3.3	1.9	1.0	0.5	0.2
Difference	+0.2	-0.5	-1.0	-0.9	-0.3	-1.1	+0.6	-0.2	-2.0	-0.5	-0.1	-1.5	-1.0	+2.2	-0.6	+1.9	-0.3	-1.9	+1.0	-0.5	-0.2

Application of Gauss' Formula:

$X = pH$

$y = \text{Frequency}$

$h = \text{parameter}$

$e = 2.718$

$\pi = 3.142$

$$y = \frac{h}{\sqrt{\pi}} \cdot e^{-h^2x^2}$$

$y = 100$ million

∴ Carex curvula should occur in a soil of pH 7.0 once in 100 million cases. Braun-Blanquet and Jenny '26 pp. 303–304

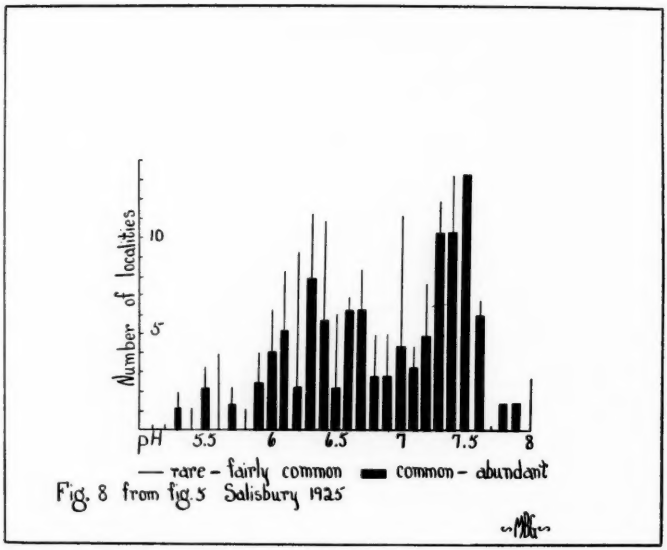


Fig. 8 from fig. 5 Salisbury 1925

species' pH are not coincident. Moreover, two or more species growing on the same soil may each have its own frequency. For example, *Pteris aquilina* and *Psamma arenaria* have respectively the modes around pH 5.5 and pH 7.1 on the same soil. He also calls attention to monomodal soils with bimodal species. Nevertheless, Pearsall (1926) offers data from English siliceous and calcareous soils intended to fill the depression of Salisbury's *Mercuriales perennis* bimodal curve and thus assume a monomodal form (see Table VI).

TABLE VI

pH Values of Soils on which Mercuriales was abundant

pH	4.5–4.9	5–5.4	5.5–5.9	6–6.4	6.5–6.9	7–7.4	7.5–8
Number of records	1	5	6	9	16	9	2
Per cent. of total number of soils	2.3	16.6	15.8	14.5	36.3	15	6.2

Influence of Plants on pH

Unless neutralizing bases are at hand plants themselves acidify the soil by their excretions, remains and selective removal. Such an increase in acidity may be quite an incidental consequence of plant growth and presence. Arrhenius (1926), though, takes a rather teleological point of view, saying that plants themselves change the substratum to a pH most suitable to themselves. Chodat (1924) in the way of opposition states that if anything one plant association may create its own downfall by making the reaction more favorable for successors. This latter point of view is in harmony with the successional studies of Salisbury (1921) and Braun-Blanquet and Jenny (1926). Quite apropos here is the work of Beauverie and Martin-Rosset (1926) who found that raised or heaped up neutral (pH 7.0) *Carex* peat is populated by a flora less specialized for marshes (amphibious and even strictly land plants) which acidify the peat and prepare it for forest species. In Lac des Echets, France, for example, the peat was removed and

otherwise modified from 1481 to the nineteenth century so that it is no longer marsh, but on the contrary now developed into forest prairies and even suitable for cultivation. This peat originally around pH 7.0 is now pH 6.0–6.2. The writer (1928) has shown that *Sphagnum* and other mosses, especially *Aulacomnium palustre*, increase the acidity of bogs. The fact that these mosses are responsible for the acidity in which they grow could very well be fortuitous. It is very clear, however, that the acidity thus produced results in the invasion of heaths and a few other species usually associated with acid habitats.

pH and Species Characters

Clausen (1922) found that *Viola tricolor* and *Viola arvensis* grow typically on acid and alkaline soils respectively. In circumneutral soils he found both species and various intermediate taxonomic forms (see Table VII).

Table 7.

Reactions of Soil

	basic	faintly acid	neutral	neutral to faintly acid	acid
No. of habitats with: Viola arvensis	7	3	8	5	
No. of habitats with: V. tricolor			1	4	4

Basic soil: small, yellowish white flowers – pure arvensis

Acid ground: large, blue, white, yellow, rose, and purple flowers – pure tricolor

Neutral-Faintly acid: pure arvensis, pure tricolor, and intermediates

From Clausen on Viola spp 1922.

pH and Concomitants

From the cases just gone over it is perfectly clear that pH is a factor operative in plant distribution. At the same time, all authorities mentioned in this paper agree, by word or act, that other factors of the soil and atmos-

phere must not be minimized or ignored. Obviously, types of soils, light and water relations are paramount. But such a general statement is a platitude. Still, anything more specific approaches speculation, for there is little in the way of precise data concerning factors attending the reaction. The difficulty of measuring these concomitants and reducing them and pH all to a common denominator makes a clear picture of the soil impossible. And yet, as Pearsall (1926) would say, an ignorance of them does not rule them out of consideration. In the following a few circumneutral relationships will be presented.

pH constant but other factors variable.—In working out his frequency tables Olsen (1921) recognized the potency of other factors. He was therefore careful to select a locality where all conditions like soil, water and light were relatively constant. In this way he proved a correlation between hydrogen-ion concentration and plant distribution. One can reverse the choice by selecting localities where the pH shall be constant, but water, light and soil factors shall vary. For an illustration see the following circumneutral habitats: (1) a dry, unstable, foredune with intense light, supporting a pioneer association; (2) a moist, stable, shady dune, dominated by a climax forest and undergrowth, Kurz (1923); (3) a raw peat with pioneer aquatics or subaquatics; (4) an old, well-decomposed peat or muck with forest trees and undergrowth, Kurz (1923 and 1928), and (5) Montfort and Brandup (1927) for a circumneutral salt marsh with its halophytic species. Atkins (1922) and later Kurz (1928) call attention to the fact that acid clays and acid sands may differ remarkably in their species.

Kotilainen on concomitants.—Kotilainen (1927) found good correlation between pH and plant distribution, yet he considers pH as a secondary, and sometimes even an unimportant, factor. Even though a certain vegetation is characterized by certain acidity it must nevertheless be understood, he holds, that acidity—itself an indicator of

other edaphic conditions—although an important factor is only one of all factors that influence species. Relation between acidity and vegetation will never be clear until pH is studied as a partial factor of a factorial complex. He admits the influence of pH, but, in contradiction especially to Arrhenius, Olsen and Chodat, thinks its influence is an indirect one. Criticizing in particular Olsen's culture work, he says that by changing acidity with the use of calcium carbonate one changes also other factors besides hydrogen-ion concentration. Olsen has stated that poverty of minerals is not important in his cases, for he found both acid and alkaline soils poor in minerals and the vegetation was totally different in the two impoverished soils. Kotilainen taking the opposite view maintains that species of *Sphagnum* can stand alkaline soil water if it is poor in nutrient salts. He states that water level, electrolyte concentration, calcium-ions, oxygen content of superficial water as well as top form of moors are often more direct factors than acidity, for these often are the very producers of acidity. Kotilainen ties up weak acidophiles with high electrolyte concentration, especially lime. Such acidophiles occur on thin layers of peat near a calcium substratum and on edges of moors. In the species *Carex capillaris, Cypripedium calceolus, Polygonum bistorta, Ranunculus propinquis* and *Epipactis palustris,* he sees a correlation between low acidity and high calcium-ion concentration. Because of their seeming dependence on calcium-ion he thinks that they had better be considered by the older concept of calciphiles. Another example: *Sphagnum papillosum* and *S. compactum* are hygrophiles and acid-loving species. In a wet habitat of pH 5.0–6.0 and a calcium salt content of 1–2 per cent. he finds them excluded. Here the acidity is proper but these species of *Sphagnum* are excluded by the calcium-ion. If hummocks form so that leaching and drying take place locally, more xerophytic acidophiles come in. Here then water content and calcium play the important rôles. Having reference to Olsen's work in

particular, page 180, Kotilainen suggests that changing the reaction by adding acids and calcium carbonate might also bring about other changes in the "factorial constellation." He admits the influence of acidity on plants if all factors are constant as Olsen has shown, but he doubts that it is a direct influence of acidity.

Salt and water content as concomitants.—Montfort and Brandrup (1927) have come to very suggestive conclusions regarding the distribution of salt marsh plants. Their work indicates that other factors outweigh the influence of pH. For instance, in Table VIII the pH is

Table 8.

Habitats	pH	Water Content of Soil %	Salt Content of Soil %
1. Meadow without Salt Vegetation on edge of Phragmites-Station	6.8	86	0.1
2. Phragmites-Station without Aster	7.0	75	0.13
3. Aster Vegetation	6.9	80	0.65
4. " "	7.1	55	0.62
5. " "	7.1	35	1.9
6. Triglochin-Vegetation	7.1	50	2.8
7. Without Vegetation	7.1	20	18.0!

From Montfort and Brandrup '27

relatively constant for all habitats. It will be seen, though, that there is an enormous variation of total salt and water content and that different types of vegetation are tied up with salt and water content of the soil. These workers conclude that *Aster tripolium* is sensitive to a high salt content and *Salicornia* to a high chlorine-ion sulphate-radical ratio. Coupled with such peculiarities of species is the fact that some of these halophytes are most sensitive to fluctuations in salt concentration as seedlings. Montfort and Brandrup make the point then that any one of a whole set of conditions, by no means static, might swing the balance in the matter of successful establishment of salt marsh species. Only a comparison of the springtime germinating conditions with those

of the summer gives an adequate understanding of the vegetational zones that correspond with gradations of salt.

Antagonism between hydrogen-ion concentration and salt concentration.—Åslander (1929) explains in a unique way the presence of neutral or alkaline soil weeds like *Chenopodium album, Sonchus oleraceous, S. arvensis, Cirsium arvense, Stellaria media* and others on acid, manured fields of northern Sweden. His experiments show that barley, a neutral soil plant, will grow in nutrient solutions with acidity as high as pH 3.75 provided only that the solution be a concentrated one as to salts. He attributes the good growth of barley in such a highly acid medium to the counteracting influence of the salts, for he found that if this high acidity was maintained in media about the concentration of soil solutions the growth declined and several plants died. On page 125 we read:

> This poor growth can not be interpreted as a result of starvation. In the nearly neutral solution the growth was practically unaffected by dilution up to 1/40. By more frequent changes (or perhaps less sodium content) the 1/80 solution would probably have been found just as suitable as the more concentrated solutions. The result can not be interpreted in more ways than one. *There is a clear antagonism between hydrogen-ion concentration and salt concentration.* No other interpretation seems possible.

Applying the information of these experiments he concludes (page 135) that the highly concentrated soil solution of newly manured fields "is responsible for the invasion of these acid soils by plants which grow ordinarily only in neutral or alkaline soils." He states further (page 136) "that the nature of the soil solution greatly affects the soil reaction at which plants thrive in nature."

pH and nitrogen content.—The importance of nitrogen content as a factor of the soil is quite generally recognized by all ecologists. However, it is difficult to study and state the exact influence of a compound, so fluctuating as to quantity and chemical constituency as nitrogen con-

tent of the soil, on plant distribution. The reader is referred to Olsen (1921), Arrhenius (1920), Hesselman (1926), who have made some correlative studies between nitrogen content, pH and plant distribution. Wherry (1926) has fallen back on total nitrogen content to explain certain pH anomalies. On Mount Desert Island, for example, a high nitrogen acidity ratio accounts for the scarcity of jack pine. The reaction pH 4.6 is suitable but the total nitrogen content too high for this pine. By a similar logic Wherry would account for the presence of certain typically acid plants in an alkaline substratum. Reference has already been made to Trümpener's (1926) conclusions about the importance of nitrogen content of the substratum for lichens. Braun-Blanquet and Jenny studied a number of depauperate and normal individuals of *Carex curvula* with reference to both pH and humus (nitrogen). They found that the species' optimum range, pH 4.6–5.6, embraced both normal and depauperate plants. But the percentage of humus ran from 34.7 to 59.0 for the normal individuals and from 12.4 to 24.7 per cent. for the depauperates. Assuming that humus content parallels nitrogen content they conclude that nitrogen is the limiting factor.

pH and salt or ionic ratios.—A number of investigators have offered various types of chemical ratios as possible adjuncts to or even substitutes for hydrogen-ion where the latter in itself seems to fail as a satisfactory explanation. Thus Pearsall (1926) advances his basic ratio hypothesis. According to him the tissues of heaths and other acid plants are rich in fats. This presence of fats suggests a soil with a high proportion of potassium and sodium to calcium and magnesium. The essence of this theory may be quoted from his article:

> We may thus find healthy species growing profusely on soils whose pH values lie between 6.0–7.0. In order to explain cases of this kind the writer suggested in 1922 that soil sourness might be attributed (*a*) to a deficiency in the soil and (*b*) to a high proportion of potassium and sodium to calcium (and magnesium).

In some of Braun-Blanquet and Jenny's soil profiles of the Alps they find that the leached horizon sometimes presents a magnesium oxide/calcium oxide ratio which is greater than unity, because calcium is leached faster than magnesium. Such soils, they state, are said to be poisonous to plants and to restrict their growth. In connection with this paragraph, Wherry's nitrogen/hydrogen-ion and Montfort and Brandrup's chlorine/sulphate ratios should be recalled. The conclusions about electrolyte content and chemical ratios are admittedly preliminary and as yet in part speculative. Nevertheless their very presentation is highly suggestive and will undoubtedly lead to a more general and thorough attack on concomitants.

SUMMARY

All workers agree that reaction is a factor in plant distribution. In cases it is undoubtedly the most important factor—notably in the *Ericaceae, Orchidaceae* and many other species. Many species are restricted to habitats of narrow reaction ranges. In fact, the correlation between distributions of species and pH may be so close that the pH data may be presented in the form of the well-known frequency curves of statisticians. Indeed, by making reverse use of such close correlations certain investigators can predict the pH accurately by means of the vegetation. It has also been shown that certain soils and vegetation develop in orderly succession toward an acidic climax vegetation on an acidic climax humus soil.

In opposition to the foregoing it must also be stated that a great many species have a wide range. And even where there is a closer relationship between species and pH it need not necessarily be laid to the direct influence of reaction unless all other factors are constant. Now in nature it is hard to find marked changes in pH without at the same time encountering drastic changes in physical, chemical and biological differences in soil and atmos-

phere. It has been shown that habitats of the same pH but differing in other factors show a corresponding difference in vegetation. However, the difficulty involved in measuring these other factors and reducing them to common denominators has caused us to minimize their potency and to assign a false supremacy to pH.

It is probably best to think of reaction relationships as a triangle whose apices are pH, plant species and concomitants. That reaction affects the distribution of plants is admitted. But when we can measure simultaneously all factors and arrange the data in overlapping and crossing frequency curves we shall be more informed on how pH affects the distribution of plants in nature.[2]

LITERATURE CITED

Arrhenius, O. von.
1920. "Öcologische Studien in den Stockholmer Schären," Diss. Stockholm.
1926. "Kalkfrage, Bodenreaktion und Pflanzenwachstum," Leipzig.

Åslander, Alfred.
1929. "Concentration of the Nutrient Medium *versus* its Hydrogen-ion Concentration as Manifested in Plant Growth," *Svensk Botanisk Tidskrift*, 23 (7): 96–140.

Atkins, W. R. G.
1922. "Some Factors Affecting the Hydrogen-ion Concentration of the Soil and its Relation to Plant Distribution, *Notes*, Bot. School, Trinity College, Dublin, 3: 133–177.

Beauverie, Jean, and Martin-Rosset.
1926. "Contribution a l'étude de l'influence de la concentration in ions hydrogène sur la flore des terrains marécageux," *Bull. Soc. Bot. France*, 72: 1045–1051.

Braun-Blanquet, J., and Hans Jenny.
1926. "Vegetationsentwicklung und Bodenbildung in der Alpinen Stufe der Zentral Alpen," Denkschr. Schweiz. Naturf. Ges., 63: 175–349. 1926.

Chodat, Fernand.
1924. "La concentration en ions hydrogène du sol et son importance pour la constitution des formations végétales," Thèse 748, Universitè de Genève, 1–115.

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Christophersen, Erling.
1925. "Soil Reaction and Plant Distribution in the Sylene National Park, Norway," Conn. Academy of Arts and Sciences, 27: 471–577.

Clausen, J.
1922. "Studies on the Collective Species of *Viola tricolor* L. II," *Botanisk Tidskrift*, 37: 363–416.

Coville, F. V.
1913. "The Agricultural Utilization of Acid Lands by Means of Acid Tolerant Crops," Bull. U. S. Dep't Agric. 6.

Geisler, Sylvia.
1926. "Soil Reactions in Relation to Plant Successions in the Cincinnati Region," *Ecology*, 7 (2): 163–184.

Hesselman, Henrik.
1926. "Studien över Barrskogens Humusstacke, dess Egenskaper och Bervende an Skogsvården" [The Humus Cover of Coniferous Forests], *Meddelanden Fran Statens Skogförsöksanstalt*, 22 (5): 176–506.

Kelley, A. P.
1923. "Soil Acidity, an Ecological Factor," *Soil Sci.*, 16: 41–54.

Kotilainen, Mouno, J.
1927. "Untersuchungen über die Beziehungen zwischen der Pflanzendecke der Moore und der Beschaffenheit, besonders der Reaktion der Torfbodens," Wissenschaftliche Veröffentlichungen des Finnischen Moorkulturvereins, No. 7. Helsinki.

Kurz, Herman.
1923. "Hydrogen-ion Concentration in Relation to Ecological Factors," *Bot. Gaz.*, 76: 1–29.
1928. "Influence of Sphagnum and Other Mosses on Bog Reactions," *Ecology*, 9: 56–69.

Mevius, Walter.
1927. "Naturwissenschaft und Landwirtschaft. Reaktion des Bodens und Pflanzenwachstum," Heft 11. 1–48. Verlag. F. P. Datterer et Cie. Freising-Munchen.

Montfort, Camill, and Wolfgang Brandrup.
1927. I. "Physiologische und pflanzengeographische Seesalzwirkung." II. "Ökologische Studien über Keimung und erste Entwicklungen bei Halophyten," *Jahrb. Wiss. Bot.* 66 (5): 902–946.

Olsen, Carsten.
1921. "Studies on the H-concentration of the Soil and its Significance to the Vegetation, especially to the Natural Distributions of Plants," Comptes-rendus du Laboratoire, Carlsberg, 15. No. 1: 1–166.

Pearsall, W. H.
1926. "Soil Sourness and Soil Acidity," *Journ. of Ecol.*, 14: 156–163.

Robinove, Joseph J., and C. D. La Rue.
1928. "The Hydrogen-ion Concentration of the Habitats of the Bryophytes and Pteridophytes of the Douglass Lake Region," Dep't. of Bot, U. of Mich.

Salisbury, E. J.

1921. "Stratification and Hydrogen-ion Concentration of the Soil in Relation to Leaching and Plant Succession with Special Reference to Woodlands," *Journ. Ecol.*, 9: 220–240.

1925. "The Incidence of Species in Relation to Soil Acidity," *Jour. Ecol.*, 13: 149.

Steagall, Mary Minerva.

1926. "Some Illinois Ozark Ferns in Relation to Soil Acidity," *Trans. Ill. Acad. Sci.*, 19: 113–137.

Trümpener, Egon.

1926. Über die Bedeutung der Wasserstoffionenkonzentration für die Verbreitung von Flechten," *Beih. Bot. Central Bl.*, Abt. 7, 42 (3): 321–354.

Ulehla, V.

1923. "Über CO_2- und pH-Regulation des Wassers durch einige Süsswasseralgen," *Berichte der deutsch. bot. Gesellschaft*, 41: 20–31.

Wehrle, Emil.

1927. "Studien über Wasserstoffionenkonzentrationsverhältnisse und Besiedelung an Algenstandorten von Freiburg in Breisgau," *Zeitschr. Bot.*, 19: 209–287.

Wherry, E. T.

1916. "A Chemical Study of the Habitat of the Walking Fern, *Camptosorus rhizophyllus* (L.) Link," *Journ. Wash. Acad. Sci.*, 6: 672–679.

1920. "Soil Acidity—its Nature, Measurement and Relation to Plant Distribution," Annual Report Smithsonian Institution, Publication 2622: 247–268.

1926. "Nitrogen as a Factor in Plant Distribution on Mount Desert Island, Maine," *Ecology*, 7 (2): 140–142.

THE RELATION BETWEEN pH AND AQUATIC ANIMALS[1]

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MUCH confusion has developed regarding the interrelations of living organisms and the hydrogen-ion concentration of the medium in which they live. This is especially true in the case of aquatic animals and the hydrogen-ion concentration, the pH, of the waters which they inhabit.

This confusion is due largely, first, to a lack of appreciation of the meaning of differences in pH in different kinds of waters and the differences in pH of the same waters; second, to the lack of a clear understanding of the behavior of carbon dioxide in solution in water and its effect upon the pH of the water through variations in tension, and third, to the want of a method for measuring the carbon dioxide tensions of natural waters. The fact that there has been no method for measuring carbon dioxide tensions of natural waters has compelled authors investigating the effects of variations in the carbon dioxide tensions of natural waters on aquatic animals to approximate the changes in carbon dioxide tension by measuring the change in the pH of the water in which the animals were living. This seemingly has led to further confusion.

It is my purpose to clear as far as possible these misconceptions.

The lack of knowledge of the true behavior of carbon dioxide in solution in water is shared alike by physicists, chemists and biologists. Johnston (1916), who has carried on extensive investigations on this problem, in

[1] Paper forming part of the symposium on "Hydrogen Ion Concentration" before the Ecological Society of America, meeting with the American Association for the Advancement of Science, Des Moines, Iowa, December 31, 1929.

speaking "of concentration of carbonic acid, combined and free in solution," states: "This treatment on the bases of fundamental principles enables us, as we shall see, to criticize and coordinate the numerous apparently contradictory statements recorded in the voluminous literature on this subject; for this conflict is due less to lack of care in the experimental work than to the fact that some essential factor—the importance of which, however, would not be recognized until the theory had been considered—was not adequately controlled." He further states "that we are here dealing with a fairly mobile equilibrium, and consequently that any change in the factors of the equilibrium is followed by a definite predictable change in the concentration of each of the molecular species present." That is, the principle of mass action suggested by Guldberg and Waage and the dielectric behavior of solvents worked out by J. J. Thomson and Nernst can be applied to carbon dioxide in solution in waters.

The author undertook (Powers and Bond, 1927) to collect a mass of data of pH readings under various carbon dioxide tensions of different natural waters to enable him to formulate a method by which the carbon dioxide tensions of natural waters could be determined from pH readings taken in the field. For this purpose eleven samples of waters were collected from mountain streams in the Smoky Mountain National Park and the Tennessee River system at lower elevations. These samples were stored in 6000 cc Pyrex glass flasks and designated as number 1, right fork of left fork of Alum Cave creek, 3,950 ft. elevation; number 2, left fork of left fork of Alum Cave creek, 3,925 ft. elevation; number 3, right fork of Alum Cave creek, 3,600 ft. elevation; number 4, West Fork Little Pigeon River, near Indian Gap Hotel, 2,400 ft. elevation; number 5, West Fork Little Pigeon River, Sugarland, 1,425 ft. elevation; number 6, Little Tennessee River, at mouth; number 7, Little River; number 8, French Broad River, at mouth; number 9, Holston River,

at mouth; number 10, Clinch River, and number 11, Sneed's spring. These waters ranged from so called "very acid" to very alkaline waters. pH readings were made of each water sample when aerated at twenty-five different known carbon dioxide partial pressures. La-Motte color standards and saturated solutions of indicators in distilled water were used. No corrections were made for different dilutions of samples due to the addition of different amounts of indicator solutions or for variations in pH readings due to the use of different indicators. Each sample was aerated with air of a known carbon dioxide partial pressure for a time[2] judged sufficient to approximate equilibrium. The pH of each sample was read without consulting the pH reading of the same sample of water at a lower carbon dioxide partial pressure. No pH reading after aeration of a sample was changed from the first reading despite the fact that an error was apparent after comparing the reading with that of the same water sample aerated with air having a different carbon dioxide partial pressure. It was not always apparent whether one or both of the two readings compared were in error. A first reading was considered as being more likely to be correct than a second prejudiced reading. The purpose was to accumulate a mass of data that would enable one to work out a method for field determination of carbon dioxide tensions of natural waters.

If mass action holds for the dissolved carbon dioxide, the equation

$$C_H \times C_{HCO_3} = KC_{H_2CO_3} \qquad (I)$$

holds in aerated water samples. C_H, C_{HCO_3}, and $C_{H_2CO_3}$ are the concentrations of hydrogen-ions, bicarbonate-ions and carbonic acid in the solutions, and K is the primary ionization constant of carbonic acid. It follows from

[2] At that time the author was not aware of the long period of time required to equilibrate carbon dioxide in solution.

equation (I) that the product of C_H and C_{HCO_3} increases or decreases with an increase or decrease in $C_{H_2CO_3}$. It also follows that unless the H-ions or the CHO_3-ions have been completely stabilized, both C_H and C_{HCO_3} increase or decrease with an increase or decrease in $C_{H_2CO_3}$. One can then write:

$$C_H = (KC_{H_2CO_3})^n \qquad \text{(II)}$$

and

$$C_{HCO_3} = (KC_{H_2CO_3})^{1-n} \qquad \text{(III)}$$

which is another way of saying that the product of C_H and C_{HCO_3} equals $KC_{H_2CO_3}$ and that both C_H and C_{HCO_3} increase or decrease with an increase or decrease in $C_{H_2CO_3}$, *i.e.*, $KC_{H_2CO_3}$. That is, the product of the equivalent of C_H in equation (II) and the equivalent of C_{HCO_3} in equation (III) equals $KC_{H_2CO_3}$. That is:

$$(KC_{H_2CO_3})^n \times (KC_{H_2CO_3})^{1-n} = KC_{H_2CO_3} \qquad \text{(IV)}$$

By substituting in equation (II) the value of $C_{H_2CO_3}$ which is given in the equation (Stieglitz, 1909)

$$C_{H_2CO_3} = k_{gas}P \qquad \text{(V)}$$

we then have

$$C_H = (Kk_{gas}P)^n \qquad \text{(VI)}$$

where k_{gas} is the solubility factor of CO_2, P the CO_2 tension of the liquid expressed in per cent. of an atmosphere[3] under standard conditions and n is the power ratio between C_H and $Kk_{gas}P$.

The equation $C_H = (Kk_{gas}P)^n$ which represents the relation of C_H to the carbon dioxide tension of all waters (systems) is the well-known mathematical Power Law and reduces to the linear form

$$\log C_H = n\log(Kk_{gas}P) \qquad \text{(VII)}$$

Since pH is the power (logarithm) of the H-ion concentration (C_H) we can write

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at mouth; number 10, Clinch River, and number 11, Sneed's spring. These waters ranged from so called "very acid" to very alkaline waters. pH readings were made of each water sample when aerated at twenty-five different known carbon dioxide partial pressures. La-Motte color standards and saturated solutions of indicators in distilled water were used. No corrections were made for different dilutions of samples due to the addition of different amounts of indicator solutions or for variations in pH readings due to the use of different indicators. Each sample was aerated with air of a known carbon dioxide partial pressure for a time[2] judged sufficient to approximate equilibrium. The pH of each sample was read without consulting the pH reading of the same sample of water at a lower carbon dioxide partial pressure. No pH reading after aeration of a sample was changed from the first reading despite the fact that an error was apparent after comparing the reading with that of the same water sample aerated with air having a different carbon dioxide partial pressure. It was not always apparent whether one or both of the two readings compared were in error. A first reading was considered as being more likely to be correct than a second prejudiced reading. The purpose was to accumulate a mass of data that would enable one to work out a method for field determination of carbon dioxide tensions of natural waters.

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The equation $C_H = (Kk_{gas}P)^n$ which represents the relation of C_H to the carbon dioxide tension of all waters (systems) is the well-known mathematical Power Law and reduces to the linear form

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Since pH is the power (logarithm) of the H-ion concentration (C_H) we can write

[3] In earlier work (Powers and Bond, 1927) P was expressed in Hg mm.

$$pH = -n\log(Kk_{gas}P) \qquad \text{(VIII)}$$

(For the minus sign before the n see the discussion that follows.)

When this formula was applied to determine the relation between C_H and the carbon dioxide tension of bog water, different lake waters and sea-water and to recalculate published data (Powers and Bond, 1927) it became evident that the factor Kk_{gas} varied too widely in acid, pure and alkaline waters to be accounted for through the variations in the values of k_{gas} (solubility factor, Johnston, 1916) due to differences in temperature and salt concentration. In order to correct for this variation a correcting factor, e_1, was introduced into the equation (Powers and Bond, 1928). Thus

$$pH = -n[\log(Kk_{gas}P) + e_1] \qquad \text{(IX)}$$

Or

$$pH = -n[\log P + \log(Kk_{gas}) + e_1] \qquad \text{(X)}$$

Since $\log(Kk_{gas})$ and e_1 are both unknown we can write

$$\log(Kk_{gas}) + e_1 = e \qquad \text{(XI)}$$

Substituting and rearranging we have

$$pH = -ne - n\log P \qquad \text{(XII)}$$

Since all H-ion concentrations ordinarily met with biologically are less than unity, pH is really a reciprocal logarithm (power). Thus, pH is a negative logarithm with the minus sign omitted (Sørensen, 1909). Thus, we can by changing all signs write

$$pH = ne + n\log P \qquad \text{(XIII)}$$

which is the equation of a straight line where the constant is the factor ne and n of the factor $n\log P$ the slope of the line and pH and logP are the two variables. In light of the revised formula all data presented by Powers and Bond (1927) have been recalculated and plotted. See Table I and Fig 1. By an inspection of Fig. 1, it is seen

TABLE I

SHOWING THE pH READINGS OF ELEVEN DIFFERENT WATER SAMPLES WHEN AERATED AT TWENTY-FIVE DIFFERENT KNOWN CARBON DIOXIDE PARTIAL PRESSURES. THE PERCENTAGE OF ERROR IS GIVEN WHEN THE FORMULA, $pH = ne + n\log P$, IS USED TO CALCULATE THE CARBON DIOXIDE TENSION OF THE WATER SAMPLE FROM THE pH READING. THE ERROR IS THE ERROR WHEN TWO PLACE LOGARITHMS ARE USED WITH AN ERROR IN THE SECOND PLACE AND WHEN IN ADDITION THESE LOGARITHMS ARE DIVIDED BY n. SEE TABLE III. SEE TABLE II FOR VALUES OF n AND ne.

CO_2 tension in Hg mm.	Water Sample																					
	Number 2		Number 3		Number 1		Number 4		Number 5		Number 6		Number 7		Number 8		Number 9		Number 10		Number 11	
	pH	% error	pH	% error	pH	% error	pH	% error	pH	% error	pH	% error	pH	% error	pH	% error	pH	% error	pH	% error	pH	% error
0.29±	4.50	+227	4.96	+137	5.29	+168	5.53	+ 8	5.95	− 19	7.44	− 20	8.07	− 37	8.20	− 53	8.47	− 3	8.36	− 7	8.55	− 7
0.65±	4.68	− 68	4.95	+ 14	5.17	+101	5.42	+ 30	5.68	− 7	6.98	+ 9	7.50	+ 11	7.54	− 19	8.04	− 13	7.90	+ 23	8.30	− 25
1.00±	4.58	− 50	4.92	− 12	5.29	− 25	5.37	+ 1	5.53	− 1	6.83	± 0	7.35	+ 2	7.38	− 6	7.86	− 12	7.81	− 0	8.08	− 19
1.26±	4.50	− 18	4.77	+ 66	4.97	+ 14	5.15	+ 85	5.25	+102	6.66	+ 18	7.23	+ 24	7.27	− 1	7.74	− 8	7.45	+ 82	7.94	− 11
1.73±	4.48	− 28	4.80	+ 2	4.89	+147	5.25	− 7	5.17	+ 94	6.63	− 7	7.05	+ 20	7.17	− 9	7.53	+ 10	7.23	+122	7.83	− 16
2.07±	4.53	− 61	4.84	− 33	4.95	+ 61	5.20	− 6	5.27	+ 16	6.51	+ 3	7.07	− 4	7.10	− 10	7.47	+ 7	7.43	+ 16	7.77	− 19
2.42±	4.46	− 39	4.83	− 40	5.10	− 27	5.19	− 18	5.33	− 19	6.51	− 12	7.02	− 8	7.04	− 12	7.41	+ 5	7.43	− 2	7.75	− 28
2.76±	4.51	− 62	4.80	− 36	5.07	− 27	5.17	− 23	5.28	− 16	6.47	− 15	6.95	− 4	6.95	− 5	7.37	+ 0	7.37	+ 0	7.55	− 0
3.12±	4.42	− 33	4.79	− 40	5.12	− 48	5.15	− 25	5.24	− 15	6.45	− 22	6.87	+ 2	6.90	− 6	7.33	− 2	7.33	− 2	7.48	+ 4
3.91±	4.40	− 35	4.77	− 46	4.98	− 26	5.07	− 19	5.20	− 22	6.35	− 22	6.78	+ 0	6.82	− 10	7.25	− 6	7.27	− 10	7.41	− 2
4.62±	4.39	− 39	4.73	− 41	4.74	+ 74	5.00	− 11	5.13	− 16	6.24	− 13	6.73	− 3	6.73	− 4	7.17	− 4	7.22	− 14	7.36	− 7
5.33±	4.37	− 38	4.72	− 47	4.87	− 13	4.96	− 10	5.04	− 2	6.16	− 8	6.62	+ 8	6.65	− 0	7.09	+ 0	7.14	− 10	7.32	− 9
7.62±	4.32	− 30	4.67	− 51	4.73	+ 10	4.80	+ 16	4.90	+ 9	5.95	+ 5	6.47	+ 7	6.45	+ 12	6.90	+ 9	6.90	+ 10	7.14	− 6
9.01±	4.20	+ 64	4.57	− 26	4.80	− 31	4.74	+ 22	4.84	+ 12	5.87	+ 8	6.42	+ 2	6.40	+ 6	6.81	+ 11	6.84	+ 6	7.03	+ 2
11.22±	4.18	+ 57	4.46	− 38	4.62	+ 24	4.72	+ 11	4.83	+ 1	5.83	− 5	6.35	− 3	6.35	− 3	6.72	+ 13	6.70	+ 18	6.96	− 3
14.76±	4.20	± 0	4.52	− 24	4.70	− 34	4.66	+ 1	4.77	− 0	5.73	− 8	6.26	− 9	6.27	− 11	6.60	+ 16	6.66	− 1	6.84	− 2
18.36±	4.18	− 4	4.43	− 20	4.62	− 24	4.60	+ 2	4.66	+ 3	5.53	+ 32	6.15	− 6	6.21	− 20	6.50	+ 17	6.48	− 4	6.72	+ 3
22.00±	4.14	+ 8	4.36	+ 19	4.53	− 11	4.57	− 9	4.63	− 5	5.49	+ 7	6.06	− 3	6.14	− 22	6.45	+ 10	6.44	+ 11	6.60	+ 13
25.39±	4.14	− 2	4.34	− 2	4.48	− 9	4.50	+ 7	4.57	+ 2	5.48	− 5	5.96	+ 5	6.10	− 24	6.40	+ 3	6.42	+ 19	6.54	+ 13
29.01±	4.16	− 28	4.32	− 4	4.43	+ 2	4.47	+ 5	4.54	− 1	5.45	− 10	5.94	− 3	6.01	− 18	6.37	− 21	6.37	− 0	6.50	+ 8
32.67±	4.13	− 17	4.28	+ 9	4.41	− 0	4.47	− 7	4.53	− 11	5.38	− 5	5.88	− 3	5.96	− 18	6.33	− 23	6.32	− 1	6.43	+ 12
36.25±	4.12	− 18	4.24	+ 21	4.36	+ 11	4.40	+ 10	4.51	− 13	5.30	+ 2	5.82	+ 2	5.88	− 10	6.27	− 0	6.24	+ 7	6.43	− 0
39.91±	4.13	− 32	4.23	+ 17	4.30	+ 30	4.35	+ 21	4.48	− 12	5.28	− 2	5.81	− 5	5.83	− 9	6.23	+ 1	6.23	+ 0	6.38	+ 3
43.43±	4.11	− 25	4.22	+ 14	4.27	+ 29	4.33	+ 20	4.43	+ 4	5.25	− 4	5.73	+ 7	5.78	− 4	6.20	− 1	6.21	− 4	6.35	+ 2
50.68±	4.08	− 16	4.20	+ 11	4.27	+ 10	4.25	+ 37	4.37	+ 1	5.17	± 0	5.60	+ 24	5.71	− 4	6.15	− 7	6.15	− 5	6.35	− 13

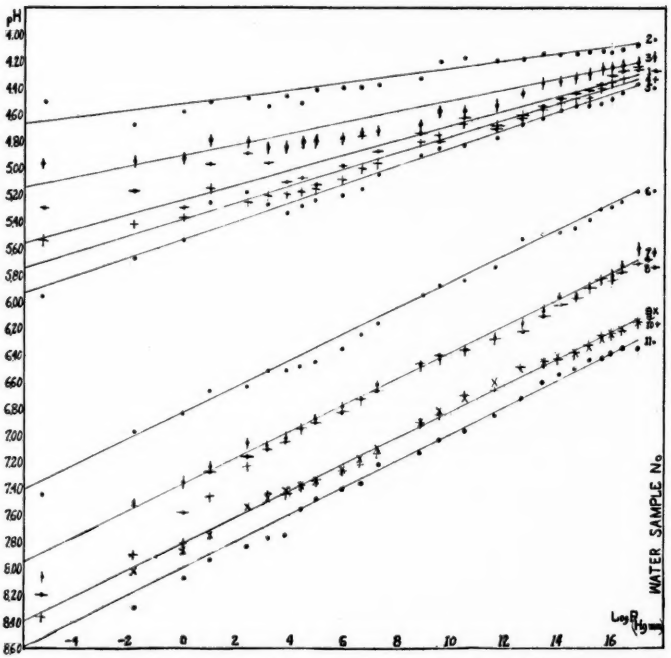

FIG. 1. A graphical representation of data given in Tables I and II.

that data of all water samples both acid and alkaline show linear relations. This is true in spite of the fact that no corrections were made for errors due to differences in the behavior of different indicators in the kinds of water and without eliminating pH readings that were obviously in error. The data of water samples numbers 3, 4 and 1 are the most irregular. These are typical mountain waters, all having been collected at an elevation of 2,400 ft. and above. In these waters there was at times a drift in the pH of an aerated sample which has not yet been accounted for. A similar rapid drift of the pH through a range of 1 to 2 has been observed in aerated seepage well waters of the Mississippi River flood plains of Arkansas. Chemists have mentioned the same difficulty when measuring the pH of lysimeter waters. A

very much less rapid but a gradual drift has been met with in other natural waters.

In the equation $pH = ne + n\log P$ the value of n decreases from the most alkaline water sample number 11, .9943, to the most acid water sample number 2, .2588.

TABLE II

SHOWING THE VALUES OF n AND *ne* FOR THE ELEVEN WATER SAMPLES. THEORETICALLY, *ne* IS THE pH OF THE WATER SAMPLE AT ONE ATMOSPHERE OF CARBON DIOXIDE TENSION.

Water sample	Value of n	Value of *ne*. pH at 1 Atmosphere CO_2 tension
2	.2588	3.7592
3	.4000	3.7477
1	.5454	3.6652
4	.6111	3.6184
5	.6762	3.5214
6	.9737	4.0249
7 & 8	.9804	4.5858
9 & 10	.9836	4.9747
11	.9943	5.1285

See Table II and Fig. 1. This merely means that the pH values of different natural waters increase at different rates with equal increase in carbon dioxide tensions. The increase is most rapid in alkaline waters and least rapid in acid waters. Theoretically, n should approach but never reach unity with increase in alkalinity. The highest value found here for n is .9943 in the most alkaline water, water sample number 11. When the carbon dioxide partial pressure equals one atmosphere logP becomes zero, then the equation $pH = ne + n\log P$ becomes $pH = ne$. Thus, *ne* is the pH of the water at one atmosphere of carbon dioxide partial pressure. As one would expect, provided the behavior of carbon dioxide is similar in solution in all natural waters, the value of *ne* decreases with the acidity of the water, there being four exceptions, water samples numbers 5, 4, 1 and 3.

Other formulas, notably that of Saunders (1926), have

been utilized for the determination of carbon dioxide tensions of natural waters. Saunders' equation, $pH = pK_1 + \log \frac{[Bik]}{[CO_2]}$, is the well-known equation of Hasselbalch. Saunders' formula can be written $pH = pK_1 + \log[Bik] - \log[CO_2]$. If for the expression $pK_1 + \log [Bik]$ we write $-ne$ and the CO_2 be transformed so as to read P, carbon dioxide tension in per cent. of an atmosphere, we have the equation, $pH = -ne - \log P$. Since pH is mathematically negative (Sørensen, 1909) we can write $pH = ne + \log P$ which is my equation (XIII) when n equals 1. The Saunders formula will not hold for acid and even slightly alkaline waters since the differences between the values of n and unity are greater than experimental error. Saunders' equation can be used in determining carbon dioxide tensions of natural waters provided n approaches so near unity that the difference is within experimental error.

From the foregoing it is evident that the pH of a natural water is merely measurement of the hydrogen-ion concentration of the water as such, and that one measurement alone tells nothing regarding the carbon dioxide tension of the water. To illustrate: if we take the carbon dioxide tension of the eleven water samples as being .316 + Hg mm ($\log P = .50000 - 1$) and inspect Fig. 1, we will find that the pH reading will be: number 11 — 8.49 –, numbers 10 and 9 — 8.3, numbers 8 and 7 — 7.85 +, number 6 — 7.31 +, number 5 — 5.86, number 4 — 5.68, number 1 — 5.50 +, number 3 — 5.10 and number 2 — 4.64. In other words, at the same carbon dioxide tension, .316 + Hg mm, the reactions of the eleven water samples vary from a very alkaline reaction, 8.49, to a very acid reaction, 4.64. For water sample number 11, a pH of 4.64 represents theoretically a carbon dioxide tension of 2.87 atmospheres. This obviously would be sufficient to kill all aquatic life. The same pH (4.64) of water sample number 2 represents a carbon dioxide tension of only .316 + Hg mm, a carbon dioxide tension that

no doubt many animals are not only able to stand but to thrive in.

All animals are essentially aquatic. All living organisms—cells—are bathed in a fluid. There are two things all organisms must accomplish if the species are to continue their existence. First, they must survive, that is, withstand their environment. And second, they must perpetuate their kind. In order to withstand their environment, nutrition and respiration must be successful. To accomplish the second, reproduction must be successful. These are axioms. If any one of these three, food gathering, respiratory exchange and reproduction, can be considered of most importance it would be the respiratory exchange, since its suspension for only a brief period is fatal to most organisms. The gathering of food can be suspended for a longer period of time. Reproduction is more or less periodic and animals can and do make longer or shorter migratory movements supposedly for the purpose of finding a suitable environment for the successful accomplishment of the perpetuation of their kind. During these migratory movements the gathering of food is more or less completely suspended and in many cases it is never again resorted to. But in all cases the respiratory exchange is continuous until the death of the animal.

When we consider the relations of aquatic animals to the physical and chemical characteristics of the waters that they inhabit we are primarily interested in a continuous successful respiratory exchange, that is, the physiology of respiration. For information regarding the physiology of respiration, including the respiratory exchange, we must go to the physiologists. The knowledge of the circulating fluid (blood of higher vertebrates) which is the transporting system in the respiratory exchange between the cells and the external environment is so great that the literature is too extensive to cite; in fact, many workers in this field consider the solution of the problem of the blood as a physicochemical system as

nearly complete or complete. For a review of the literature, see Haldane, 1927, Y. Henderson, 1925, and other good reviews in *Physiological Reviews*. The problem of the physiology of regulation of the blood is not complete. To know that there are differences of opinion among the leading physiologists regarding even the facts in the problems of regulation it is only necessary to read the above citations. In all the work on the physiology of respiration of lung-breathing animals the following facts seem to stand out (Y. Henderson, 1925): "The amount of alkali in use in the blood of a healthy individual is fundamentally regulated and determined by the pressure of oxygen in his lungs . . . the type of blood that he has in his vessels at any one time is a function of the mean barometer at the place where he is living. . . . The pressure at the new home induces a slow readjustment of the blood, requiring days or weeks to become complete." Another fact is that the carbon dioxide tension of the alveolar air is delicately regulated by the extent of ventilation of the lungs (Campbell, Douglas and Hobson, 1914). In short, the relations between the amount of alkali in use in the blood and the oxygen and carbon dioxide tensions of the arterial blood are regulated and keep lung-breathing animals in harmony with their external environment. When the external environment is changed there is a readjustment in all three. This again brings the animal into harmony with its environment. This readjustment is at first rapid. Like all reactions approaching equilibria, the rate is slowed down, a complete readjustment requiring a longer period of time. In short, lung-breathing animals adjust within limits both the internal and external environment. The foregoing statements do not raise the question as to how this readjustment is brought about but merely state the fact that there is a mechanism that tends to keep lung-breathing animals in adjustment with their oxygen and carbon dioxide external environment (the alveolae of the lungs being considered as the external environment).

Investigators of the physicochemical characteristics of the environment of aquatic animals must first determine the probability of the presence of a mechanism in aquatic animals similar to that of lung-breathing animals that keeps them in adjustment with the oxygen and carbon dioxide tensions of their environments—the two gases

TABLE III

SHOWING PERCENTAGE OF ERROR IN CALCULATING THE CARBON DIOXIDE TENSION WITH AN ERROR IN pH READING

Water sample	pH error of .01	pH error of .10	Water sample	pH error of .01	pH error of .10
No. 2	+9.5 to −8.5	+143.0 to − 59.0	No. 5	+3.5 to −3.3	+41.0 to −29.0
No. 3	+6.0 to −5.6	+ 78.0 to − 44.0	No. 6	+3.0 to +2.4	+21.0 to +26.0
No. 1	+4.3 to −4.0	+ 52.0 to − 44.0	11	−2.5 to −2.2	−21.0 to −20.0
No. 4	+4.0 to −3.7	+ 46.0 to − 31.0			

concerned in the respiratory exchange—in order to determine the most important environmental factors to investigate.

It must first be determined whether or not there exists a circulatory fluid—an oxygen and carbon dioxide transporting system—possessing physicochemical characteristics comparable to those of the blood of lung-breathing animals. That is, are the bloods (circulatory fluids) of aquatic animals and the bloods of lung-breathing animals comparable physicochemical systems? For the answer to this question we again go to the physiologists. The work of Krogh and Leitch (1919) and Wastl (1928) shows that the oxygen dissociation curves of blood of fishes are

characteristically comparable to those of the blood of lung-breathing animals. The findings of the investigations on the hemocyanins may be summarized as follows. (Parsons and Parsons, 1923): ". . . the blood proteins act as weak acids and expel carbon dioxide from the blood at low tensions which include the physiological range just as in vertebrates the hemoglobin similarly displaces carbonic acid from its combination." (Redfield, Coolidge and Hurd, 1926): ". . . it is shown that the properties of the hemocyanin of each of these species are distinctive, but that all these proteins function in the transport of oxygen and carbon dioxide according to the same physicochemical principles as obtained in the case of hemoglobin." (Redfield and Hurd, 1925): ". . . the effect of carbon dioxide upon the oxygen dissociation curve is similar to that observed by Bohr, Hasselbalch and Krogh in the case of human blood." (Hogben and Pinhey, 1927): "The different values of the 'critical pH' found for haemocyanins of different crustacea . . . indicate that even the haemocyanins of different species of decapod crustacea are different." (Stedman and Stedman, 1928): Haemocyanin has an oxygen solubility curve at which it is 97½ per cent. saturated at about 150 mm, *i.e.*, when in equilibrium with the air. (Redfield and Hurd, 1925): "It is suggested that there may be a relationship between the tensions at which the tissues of an organism utilize oxygen to best advantage and the pressure at which its respiratory proteins can carry on oxygen exchange." Furthermore, the work of Powers, and Powers and Logan (1925) on the effect of carbon dioxide and oxygen tensions of sea water on the alkali reserve of the blood plasma of certain marine fishes, and of Hall (1926) showing that lowering oxygen tension increases the number of red blood corpuscles of the seventeen species of marine fishes tested, all shows that the relation of the blood of aquatic animals, of at least marine fishes, if not the same is at least comparable to that of the higher vertebrates. It follows that

the carbon dioxide and oxygen tensions of natural waters are important factors to be considered in any attempt to analyze the relations between the physicochemical conditions of the water and at least those aquatic animals that carry oxygen transporting proteins in their blood.

Since all animals are essentially aquatic the fluid lining the alveolar membranes of lung-breathing animals must have a hydrogen-ion concentration modified by the carbon dioxide partial pressure of the alveolar air. All facts regarding the pH and osmotic pressure of this fluid are unknown. Consideration of osmotic pressure is without the scope of this paper.

Aquatic animals, unlike lung-breathing animals, are not so well able to control the carbon dioxide and oxygen tensions of their external environment. Their gills are always more or less exposed to the actual carbon dioxide and oxygen tensions of the environment. Regulation must depend to a greater extent upon the blood itself or the animal must be able to withstand greater variations in its blood when it meets with variations in the physicochemical conditions of the water. This regulation must be rapid where there is a rapid change in the carbon dioxide or oxygen tension, or both. The more rapid the regulation the more independent the animal is of the external carbon dioxide and oxygen environment and perhaps the wider its distribution. The less rapid the regulation the more dependent the animal is upon the conditions of the environment and the less wide its distribution, and the greater the tendency to remain in the same kind of water to which it is adjusted. The behavior of the animal to its environment then becomes of prime importance.

Shelford and Allee (1913) plainly show that fishes do react to differences in carbon dioxide (tension) and that the reactions to differences in oxygen content (tension) were in general very indefinite—findings one would expect from what has been brought out in the foregoing discussion. These same authors in a preliminary note

suggested that "the carbon dioxide content (strong alkaline waters excepted) is probably the best single index of the suitability of that water to fishes." I have shown that crayfishes reacted negatively to higher concentrations (tensions) of carbon dioxide. Attention was also called to the fact that the crayfishes were more sensitive to carbon dioxide than to acidity. In later investigations —work done at a time that Sørensen's (1909) classic work was becoming well known and at a time that the works on the blood as a physicochemical system were obtaining prominence though not as well understood then as now—it is not clear (Shelford and Powers, 1915, and Shelford, 1918) whether the fish were reacting to pH as such or to the carbon dioxide tension of waters except when the higher acidities were brought about by the actual addition of carbon dioxide. In those cases the fish (herring) were always negative to the higher concentrations (tensions) of carbon dioxide. In 1923 Shelford ran an extensive series of experiments in which he found that all species of fresh-water fishes tested responded to differences in hydrogen-ion concentrations. Later Shelford (1929) in speaking of these experiments states[4]: "With any given temperature, salt content, etc., they usually behave consistently, and with any one set of conditions could be depended upon to select a given hydrogen-ion concentration; but as conditions were varied and the number of readings increased the results vary, indicating that control lay with another factor. . . . Na_2CO_3 shifts the selection to lower hydrogen-ion content," a fact that one would expect if the fishes were selecting definite ranges in carbon dioxide tensions. In spite of the fact that a reanalysis of experiments indicates that the fishes were reacting to carbon dioxide tensions rather than to pH as such at this time, 1915–, it was the general opinion of most investigators that pH as such was of prime im-

[4] Shelford's book, "Laboratory and Field Ecology," 1929, gives one of the best discussions in print of the interrelations of aquatic animals and the physicochemical conditions of the water.

portance. This, as has already been stated, was due to the lack of an accurate conception of the behavior of carbon dioxide in solution which could not be appreciated until the theory had been worked out by the chemists. In 1909 Stieglitz contributed to a better understanding of this problem. Stieglitz's paper, one of these hidden publications, was not well known even to chemists for some time. Johnston and coworkers (1915 and 1916) and Walker, Bray and Johnston (1929) have further contributed to the knowledge of the behavior of carbon dioxide in solution. The solution of the problem is not yet complete. In 1915 Johnston states, "The value of n" (in his equation $[H_2CO_3]/[CO_2]_s = n/(1 - n)1$) "is not known." Later Walker, Bray and Johnston (1929) state, "This mode of treatment, in terms of activity instead of concentration, obviates the necessity of taking into account the proportionate hydration of carbon dioxide in solution . . ." Since it is the tension of the carbon dioxide that reaches equilibrium on the two sides of a permeable membrane and not the absolute number of molecules, the knowledge of the value of n is not important when considering the diffusion of carbon dioxide into and out of the blood of aquatic animals. However, to obtain a clear understanding of the behavior of carbon dioxide at the same tension in different kinds of solutions, a knowledge of the value of the ratio between dissolved carbon dioxide and the hydrated carbon dioxide becomes essential. It is the lack of this knowledge that has retarded for so long an appreciation of the relation of pH and the carbon dioxide tensions of different natural waters—an appreciation which is still more or less lacking in certain chemists who are investigating the chemistry of natural waters.

In reviewing the literature too extensive to cite on the relation of pH and various physiological activities, and the behavior and distribution of aquatic animals, it becomes difficult to determine whether or not the prime factor is pH as such or some other factor or factors as-

sociated with the variation in pH. The author wishes here to sound a warning against any attempt to explain any physiological activity, behavior, or the distribution of animals by any one factor. He wishes further to emphasize the fact that behavior or distribution is not a matter of tolerance alone; Shelford has pointed out the necessity of a quantitative study of animal distribution and not the extent of distribution in the study of marine and fresh-water communities.

When considering the effect of oxygen tension on the rate of oxygen absorption it is found with few exceptions that the rate is not retarded materially until comparatively low tensions are reached. There are exceptions. Amberson, Mayerson and Scott (1924), working on marine invertebrates, and Nomura (1926) working on *Caudine* found the oxygen absorption to be directly proportional to the oxygen tension of the water. Hall (1929) tested three species of marine fishes. The scup showed very little change in oxygen consumption with change in oxygen tension. The puffer showed a slightly greater change. But in the toadfish the oxygen consumption was almost directly proportional to the oxygen tension. Amberson, Mayerson and Scott state, "We have satisfied ourselves that this diminution in oxygen consumption with lowered oxygen tension is not due to the accumulation and inhibitory action of CO_2 in the water . . ."

The results of the investigations on the effect of carbon dioxide (total amounts of carbon dioxide being excepted) are more consistent. Many workers have shown that physiological and body processes were affected by carbon dioxide. Pruthi found an increase in carbon dioxide more detrimental than variations in pH or decrease in oxygen content. Hyman (1925) has found the depression of oxygen consumption in *Planaria* "to be due chiefly or wholly to the CO_2 and not to acidity"—pH. Pereira (1926) found oxygen consumption of sea-water fishes depressed by increased

carbon dioxide tension if we assume that the decrease in pH meant an increase in carbon dioxide tension. Powers and Shipe (1928) attempted to determine the effect of carbon dioxide tension and oxygen tension on the rate of oxygen consumption by marine fishes before they had become adjusted through regulation. They exposed the fishes to various carbon dioxide and oxygen tensions and determined the rate of oxygen consumption during the first ten minutes. They found that carbon dioxide did affect the rate of oxygen consumption. A combination of low oxygen and high carbon dioxide was more effective than either low oxygen and low carbon dioxide or high oxygen and high carbon dioxide. The herring was most sensitive, the silver salmon or coho next and the viviparous perch least. Their findings were in keeping with the habits of these three species of fishes. Powers (1922) found that the lowest oxygen concentration (tension) at which fishes could absorb oxygen was affected by the pH of the sea water. There being no rapid method at that time to determine the carbon dioxide tension of waters Powers took the pH of the water in this and other investigations as the best measurement of carbon dioxide tension (McClendon, 1917). This, and especially since Cameron and Mounce (1922) had found herring at a different pH from that found by Powers (1921), has led to conflicting statements by Jobes and Jewell (1927) and Thompson, Miller, Hitchings and Todd (1929). This was in spite of the fact that Powers suggested in these papers that carbon dioxide tension was the probable factor. The data in Table I (Powers and Logan, 1925) are arranged in decreasing carbon dioxide partial pressures of the air used for hours to aerate the water in which the fish were placed. Air of different carbon dioxide partial pressures was resorted to, since the carbon dioxide tension of the water could not be measured rapidly. The data can not be rearranged as in

Table II (Jobes and Jewell, 1927) according to the titratable alkalinity (Johnston, 1916) and indicate the comparative carbon dioxide tensions accurately by the pH of the water. See Fig. 1 and Table I of this paper. If the difference of .22 of a pH found by Thompson, Miller, Hitchings and Todd (1929) in the margins of two opposing tidal currents were due to differences in carbon dioxide tensions alone, *i.e.,* had the waters been the same the carbon dioxide tension in the one would have been 1.69 times as high as the other, taking the value of n found by Powers for certain Puget Sound waters or 1.66 times if Saunders' equation is applied. Or if the principle of the formula of Thompson, Miller, Hitchings and Todd is used in the calculation the difference will be 1.75 + times. This difference in carbon dioxide tension would have been sufficient under ordinary conditions to have reduced the capacity of the herring to absorb oxygen to less than one third during its first ten minutes of sojourn (Powers and Shipe, 1928). This no doubt the herring would have felt and turned back or would have sought deeper waters, since herring do react to less differences in carbon dioxide tensions (Powers, 1921). It is common knowledge that the appearance of herring around the Biological Station at Friday Harbor is erratic. The probability of marked physiological effects brought about by a sudden change of 1.66 to 1.75 + times the carbon dioxide tension of the external environment of a fish is further emphasized by the fact that an increase of .22 per cent. in the alveolar carbon dioxide tension, the external environment of man, is sufficient to double the alveolar ventilation (Campbell, Douglas and Hobson, 1914). Thus an increase of 66 to 75 per cent. in carbon dioxide tension is 30 to 340 times that necessary to increase the alveolar ventilation in man 100 per cent. If the difference in pH at Friday Harbor where herring were found most abundant by Powers (1921), pH 7.7 to 7.9, and near Nanaimo where herring are known to occur in water varying from pH 8.3 to 8.8 (Cameron and

Mounce, 1922) were due to change in carbon dioxide tension alone[5] the carbon dioxide tension of the water near Friday Harbor would be from 3.98 to 12.5 times as great as at Nanaimo, a carbon dioxide tension difference no doubt sufficient to cause marked physiological changes in all fishes in the sound if its carbon dioxide tension were to be thus suddenly changed. As has been stated, behavior is not altogether a matter of tolerance or even regulation but all knowledge of the physiology of respiration and of the blood as a physicochemical system tells us that the animal must and does regulate to a change in carbon dioxide tension and oxygen tension of the external environment. Differences in carbon dioxide tensions have been found in natural waters by Shelford (1925), Powers and Hickman (1928) and Powers (1928) in fresh waters; Krogh (1904) in fresh and marine waters, and Moore, Prideaux and Herdman (1915), Henderson, Cohn, McClendon, Gault, Mulholland and Mayer in oceanic waters. A question in aquatic ecology is, do fishes regulate suddenly and move about regardless of the kind of water that they enter, or is the reaction of the more so called sensitive fishes to the kinds of water a part of the regulatory mechanism? It is common knowledge that the red salmon do choose between two waters (rivers) when a choice is presented. River waters at their junction do differ in pH, carbon dioxide tension, oxygen tension and temperature (Ward, 1921 [temperature], Shelford, 1925 [pH and temperature] and Powers and Hickman, 1928, and Powers, 1928 [pH, carbon dioxide and oxygen tensions and temperature]). It has long been known that herring do behave as they would if they were reacting to differences in different oceanic waters (Pettersson, 1894 and 1921).

It is the author's conclusion that carbon dioxide tension is an important factor, but not the only factor, in the distribution and migratory movements of aquatic

[5] In this and the Thompson and Miller findings, the actual differences in carbon dioxide can not be determined from the data given.

animals, especially the migratory fishes. Temperature no doubt is a factor (Ward, 1921). Temperature is no doubt a factor as such and in addition temperature changes affect both the pH and carbon dioxide tension of water provided temperature change is the only change taking place in the water.

It is hoped that the foregoing discussion will tend to clear the apparently contradictory findings recorded in the extensive literature on the pH of natural waters and its relation to aquatic animals.

SUMMARY

1. The pH of a natural water is determined by the temperature, solids in solution and its carbon dioxide tension.

2. A change in the carbon dioxide tension of a water causes a definite and predictable change in pH, provided the carbon dioxide in solution is the only factor modifying the system. The amount of change in pH with a given change in carbon dioxide tension is determined by the water itself as a chemical system. That is, the pH at a definite carbon dioxide tension and temperature and the amount of change with a definite change in carbon dioxide tension are determined by the characteristics of the natural water itself.

3. Aquatic organisms are able to withstand wide ranges in pH.

4. Aquatic animals are affected in their physiological processes and behavior by changes in carbon dioxide and oxygen tensions. Of the two, a change in carbon dioxide tension is the more effective.

5. All aquatic animals can regulate their internal environment to bring them into harmony with their external environment. The range and rapidity of this regulation are characteristics of the species and are no doubt factors in determining the distribution and the movements of migratory animals.

6. Nothing is said regarding the physiology of regulation, that being without the scope of this paper.

LITERATURE CITED

Amberson, W. R., H. S. Mayerson and W. J. Scott.
1924. "The Influence of Oxygen Tension upon Metabolic Rate in Invertebrates," *Jour. Gen. Physiol.*, 7: 171–176.

Cameron, A. T., and I. Mounce.
1922. "Some Physical and Chemical Factors Influencing the Distribution of Marine Flora and Fauna in the Strait of Georgia and Adjacent Waters," *Contrib. Can. Biol.*, n. s., 1: 39–72.

Campbell, J. M. H., C. G. Douglas and F. G. Hobson.
1914. "The Sensitiveness of the Respiratory Centre to Carbonic Acid, and the Dead Space during Hyperpnoea," *Jour. Physiol.*, 48: 303–316.

Haldane, J. S.
1927. "Acclimatisation to High Altitudes," *Physiol. Reviews*, 7: 363–384.

Hall, F. G.
1929. "The Influence of Varying Oxygen Tensions upon the Rate of Oxygen Consumption in Marine Fishes," *Amer. Jour. Physiol.*, 88: 212–218.

Hall, F. G., I. E. Gray and S. Lepkoosky.
1926. "The Influence of Asphyxiation on the Blood Constituents of Marine Fishes," *Jour. Biol. Chem.*, 67: 549–554.

Henderson, Y.
1925. "Physiological Regulation of the Acid-base Balance of the Blood and Some Related Functions," *Physiol. Reviews*, 5: 131–160.

Hogben, L. T., and K. F. Pinhey.
1927. "Some Observations on the Haemocyanin of *Limulus*," *Brit. Jour. Expt. Biol.*, 5: 55–165.

Hyman, L. H.
1925. "On the Action of Certain Substances on Oxygen Consumption. VI. The Action Acids," *Biol. Bull.*, 49: 288–322.

Jobes, F. W., and M. E. Jewell.
1927. "Studies on the Alkali Reserve of the Blood of *Ameriurus nebulosus* from Acid and Basic Waters," *Trans. Amer. Micros. Soc.*, 46: 175–186.

Johnston, J.
1915. "The Solubility-product Constant of Calcium and Magnesium Carbonates," *Jour. Amer. Chem. Soc.*, 37: 2001–2020.
1916. "The Determination of Carbonic Acid, Combined and Free, in Solution, Particularly in Natural Waters," *Jour. Amer. Chem. Soc.*, 38: 947–975.

Krogh, A.
1904. "On the Tension of Carbonic Acid in Natural Waters and Especially in the Sea," *Meddelelser om Grønland*, 26: 333–405.

Krogh, A., and I. Leitch.
1919. "The Respiratory Function of the Blood in Fishes," *Jour. Physiol.*, 52: 288–300.

Mayer, A. G.
1919. "Detecting Ocean Currents by Observing their Hydrogen-ion Concentration," *Proc. Amer. Phil. Soc.*, 58: 150–160.

McClendon, J. F.
1917. "The Standardization of a New Colorimetric Method for the Determination of the Hydrogen-ion Concentration, CO_2 Tension, and CO_2 and O_2 Content of Sea Water, of Animal Heat, and of CO_2 of the Air, with a Summary of Similar Data on Bicarbonate Solutions in General," *Jour. Biol. Chem.*, 30: 265–288.

Moore, B., E. B. R. Prideaux and G. A. Herdman.
1915. "Studies of Certain Photo-synthetic Phenomena in Sea-water. I. Seasonal Variations in the Reaction of Sea-water in Relation to the Activities of Vegetable and Animal Plankton. II. The Limitations of Photo-synthesis by Algae in Sea-water," *Trans. Biol. Soc. Liverpool*, 29: 171–202.

Nomura, S.
1926. "The Influence of Oxygen Tension on the Rate of Oxygen Consumption in Caudina," Sci. Repts. Tohoku Imp. Univ., 4th Ser., Biol., Sendai, Japan, 2: 133–138.

Parsons, T. R., and W. Parsons.
1923. "Observations on the Transport of Carbon Dioxide in the Blood of Some Marine Invertebrates," *Jour. Gen. Physiol.*, 6: 153–166.

Pereira, J. R.
1926. "On the Influence of the Hydrogen-ion Concentration upon the Oxygen Consumption in Sea-water Fishes," *Biochem. Jour.*, 18: 1294–1296.

Pettersson, O.
1894. "A Review of Swedish Hydrographic Research in the Baltic and North Sea. V. The Waters in the Swedish Fiords and in the Submarine Ponds and Channels of the Coast-changes in the Coast-water at the Close of the Winter Season, and their Effect upon the Herring Fishery," *Scot. Geog. Mag.*, 10: 525–539.
1921. "Étude sur les mouvements internes dans la mer et dans l'air," *Svenska Hydro.-Biol. Komm. Skrifter*, VI.

Powers, E. B.
1921. "Experiments and Observations on the Behavior of Marine Fishes toward the Hydrogen-ion Concentration of the Sea-water in Relation to their Migratory Movements and Habitat," *Pub. Puget Sound Biol. Sta.*, 3: 1–22.
1922. "The Physiology of the Respiration of Fishes in Relation to the Hydrogen-ion Concentration of the Medium," *Jour. Gen. Physiol.*, 4: 305–317.
1928. "The Carbon Dioxide Tension, Oxygen Content, the pH and the Alkali Reserve of Natural Waters Mostly of the Western Portion of the United States," *Pub. Puget Sound Biol. Sta.*, 5: 381–391.

Powers, E. B., and J. D. Bond.

1927. "A Simple Colorimetric Method for Field Determinations of the Carbon Dioxide Tension and Free Carbon Dioxide, Bicarbonates and Carbonates in Solution in Natural Waters. II. A Critical Mathematical Analysis of Theory and Data," *Ecology*, 8: 471–479.

1928. "Further Notes on the Colorimetric Method for Field Determinations of the Carbon Dioxide Tension of Natural Waters," *Ecology*, 9: 364–366.

Powers, E. B., and T. A. Hickman.

1928. "The Carbon Dioxide Tensions of the Fraser River and its Lower Tributaries and of Certain Tributaries of the Columbia River," *Pub. Puget Sound Biol. Sta.*, 5: 373–372.

Powers, E. B., and G. A. Logan.

1925. "The Alkaline Reserve of the Blood Plasma of the Viviparous Perch (*Cymatogaster aggregatus* Gib.) in Relation to the Carbon Dioxide Tension, the Oxygen Tension and the Alkalinity of the Sea-water," *Pub. Puget Sound Biol. Sta.*, 3: 337–360.

Powers, E. B., and L. M. Shipe.

1928. "The Rate of Oxygen Absorption by Certain Marine Fishes as Affected by the Oxygen Content and Carbon Dioxide Tension of the Sea-water," *Pub. Puget Sound Biol. Sta.*, 5: 365–380.

Redfield, A. C., T. Coolidge and A. L. Hurd.

1926. "The Transport of Oxygen and Carbon Dioxide by Some Bloods Containing Hemocyanin," *Jour. Biol. Chem.*, 69: 475–509.

Redfield, A. C., and A. L. Hurd.

1925. "The Respiratory Function of the Hemocyanins," *Proc. Nat. Acad. Sci.*, 11: 152–155.

Saunders, J. T.

1926. "The Hydrogen-ion Concentration of Natural Waters. I. The Relation of pH to the Pressure of Carbon Dioxide," *Brit. Jour. Expt. Biol.*, 4: 46–72.

Shelford, V. E.

1918. "Relation of Marine Fishes to Acids with Particular Reference to the Miles Acid Process of Sewage Treatment," *Publ. Puget Sound Biol. Sta.*, 2: 97–111.

1925. "The Hydrogen-ion Concentration of Certain Western American Inland Waters," *Ecology*, 6: 279–287.

1929. "Laboratory and Field Ecology," Williams and Wilkins Company, Baltimore.

Shelford, V. E., and W. C. Allee.

1913. "The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases," *Jour. Expt. Zool.*, 14: 207–266.

Shelford, V. E., and E. B. Powers.

1915. "An Experimental Study of the Movements of Herring and Other Marine Fishes," *Biol. Bull.*, 28: 315–334.

Sørensen, S. P. L.
1909. "Études enzymatiques. II. Sur la mesure et l'importance de la concentration des ions hydrogène dans les réactions enzymatiques," *Compt. rend. Lab.*, Carlsberg, Copenhagen, 8: 1–168.

Stedman, E., and E. Stedman.
1928. "Haemocyanin. Part V. The Oxygen Dissociation Curve of Haemocyanin from the Snail (*Helix pomatia*) in Dialysed Solution," *Biochem. Jour.*, 22: 889–901.

Stieglitz, J.
1909. "The Relations of Equilibrium between the Carbon Dioxide of the Atmosphere and the Calcium Sulphate, Calcium Carbonate and Calcium Bicarbonate of Water Solutions in Contact with it," Carnegie Inst., Washington, Pub. 107: 233–364.

Thompson, T. G., R. C. Miller, G. H. Hitchings and S. P. Todd.
1929. "Studies of the Sea-water near the Puget Sound Biological Station during the Summer of 1927," *Publ. Puget Sound Biol. Sta.*, 7: 65–99.

Ward, H. B.
1921. "Some of the Factors Controlling the Migration and Spawning of the Alaska Red Salmon," *Ecology*, 2: 235–254.

Wastl, H. A.
1928. "Beobachtungen über die Blutgase des Karpfenblutes," *Biochem. Zeit.*, 197: 363–380.

Walker, A. C., U. S. Bray and J. Johnston.
1927. "Equilibrium in Solution in Alkali Carbonates," *Jour. Amer. Chem. Soc.*, 49: 1235–1256.

THE ECOLOGICAL DETERMINATION OF TWISTED HYPOCOTYL AND OTHER PECULIAR EXPRESSIONS IN HEMP[1]

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HAVING been engaged in the experimental study of *Cannabis sativa* for the past fifteen years, especially in relation to sex control, sex reversal and repeated rejuvenations, I have naturally seen many odd character expressions which have appeared from time to time in the cultures. Some of these peculiar expressions were either mutations or Mendelian segregations, but it is not with these that this paper deals. I wish to call attention particularly to a number of extreme and unusual character expressions which do not appear when the plant is grown in the usual or so-called normal conditions. It has been found that the morphological and physiological reactions and expressions are different with every decided change in the environment.

EXPRESSION OF TWISTED AND TWINING HYPOCOTYLS

When hemp is grown in a long light period the hypocotyl is comparatively short and straight, but if it is grown in a short-period day of eight or nine hours' duration and the light is at the same time low in intensity the hypocotyl elongates enormously the first ten to fourteen days after the plant emerges from the ground, often becoming five to seven inches long before any internode develops in the plumule and at the same time the first pair of leaves expand but slightly. The plant appears to be starving to death. Now if the illumination is intensified somewhat, growth continues and the plants can be kept from dying. In a patch of hemp treated thus,

[1] Papers from the department of botany, the Ohio State University, No. 260. Delivered before the Ohio Academy of Science, Columbus, Ohio, on April 18, 1930.

a considerable number of individuals developed spiral twists in the upper part of the hypocotyl, mostly in the upper one to two inches. Sometimes, however, the twist was from near the base to the top of the hypocotyl. This torsion is quite remarkable and sometimes as many as four twists are developed. Frequently when two plants are close together they twine around each other with two or three turns when the hypocotyls are from five to seven inches long. The twining is clockwise. Thus the new environment causes entirely new morphological and physiological expressions. It would be unreasonable to say that these new reactions were caused by new genes or by latent genes which do not come to expression under the usual environment. It is the entire mechanism of the system of protoplasts that is involved in the reaction, and no more gene addition or subtraction is required to explain this remarkable elongation, torsion and twining of the stem than is required to explain why a child learns to speak perfect English in one environment and perfect Spanish or French in another.

TWO TO FOUR INTERNODES INSTEAD OF TWENTY TO FORTY

When hemp is grown in the proper short light period of winter with appropriate nutrients, heat and moisture it commonly develops but two or three internodes above the cotyledons with a very small terminal inflorescence of a few flowers. The same variety when grown out of doors in the long daylight period of spring and summer develops about twenty internodes before the inflorescence appears. If it is developed in continuous light or in a very long light period it may develop over fifty internodes with the corresponding number of leaves, thus when judged by the number of vegetative internodes produced the two extremes differ by 2,500 per cent. The character becomes such as the environment determines. A short light period usually brings the plants into bloom in three or four weeks after they emerge from the ground. I have actually seen stigmas beginning to protrude from

reversed staminate plants on the fourteenth day after the embryos came through the soil. These plants die promptly after the reproductive cycle is complete. In continuous light or in a very long daylight period the plant remains in the vegetative condition for a very long time and six months may elapse before flower development begins. Thus the length of the one vegetative period is about thirteen times as long as the other. The length of the vegetative period is largely determined by the environmental conditions.

Opposite and Alternate Leaf Arrangements

When hemp is grown in the summer in continuous light (electric light at night), a variety commonly used in the experiments changed from the opposite leaf condition to the alternate leaf condition at the seventh to fourteenth node, while the same variety grown with the usual nightly darkness of summer does not change until the twentieth to twenty-fifth node is developed. With proper photoperiodicity, hemp plants can be grown with only four opposite leaf nodes at the base and thirty or more alternate nodes developed during the remainder of the ontogeny. Thus, to mention a specific case, a staminate plant in which the leaves were three in a whorl had one whorl of cotyledons, two whorls of vegetative leaves and two whorls of bracts in the inflorescence when it was becoming senile and complete determination of the terminal bud had been attained. Since this plant was desired for further experimental purposes it was not permitted to die a peaceful, natural death but rejuvenated by means of exposure to continuous light. The terminal bud promptly continued vegetative growth and thus from the sixth node on, counting the cotyledon node, the leaves were alternate. This condition gave a very striking sudden change in character because the lower leaves were in threes.

DIFFERENCE IN SEXUAL DIMORPHISM

If hemp is grown in a comparatively short daylight period so that three to six internodes develop before the inflorescence appears, a decided sexual dimorphism develops which is especially prominent in the second internode above the cotyledons. Usually the third internode also shows the dimorphism. The second internode is commonly more than twice as long in the staminate plant as in the carpellate plant. At the time of blooming the staminate plants shoot up far beyond the carpellate plants because of the rapid elongation of their internodes. The average height of the staminate plants is about twice that of the carpellate plants, which gives the patch the appearance of a double-canopied forest. This reaction is the most pronounced if the planting is done between January 15 and February 15. In this case the plants have the advantage of the rapidly increasing light at just about the time they begin to develop flowers. Now when the plants are grown in the usual, long daylight period no such sexual dimorphism is evident in the second and third internodes, and although the plants as a whole are strongly dimorphic in various characters, the staminate plants are no taller than the carpellate, but are actually usually lower. The special sexual dimorphism is determined by the environment and the reaction is under the control of external factors, even though, as will readily be admitted, the mechanism of the hemp protoplast is of such a nature as to bring out the diverse response under the two environmental determinative conditions. The point is that the sexually dimorphic second internodes are just as normal as the homomorphic second internodes which develop in the long light environment. The plants grown in the short light can be perpetuated generation after generation just as definitely as those which are more commonly grown in the long light. The great difference in size and reaction between the long light and short light plants is thus not due to the addition or sub-

traction of hereditary units but entirely to the addition and subtraction of environmental factors.

Expression of Unisexual and Bisexual Individuals

Under the usual summer conditions the ordinary varieties of hemp grown are quite strictly dioecious, the individuals being pure male and pure female in sex expression. According to the old XX, XY formula they are so because the female is homozygous for assumed sex factors and sex chromosomes and the male is heterozygous, with the male factors dominant over the female. The chromosome complement of hemp, according to recent cytological investigations, evidently contains a set of allosomes, AA allosomes in the carpellate plants and AB in the staminate plants. Now if hemp is grown with the proper short light periodicity, all individuals both male and female become bisexual in expression, and this bisexuality of the individual can be continued generation after generation, so long as the environmental conditions are maintained that cause such a reaction, just as definitely as the unisexual condition is expressed in the long light complex. In the short light period also there will, as stated, be only two to five internodes, with the corresponding reduction in leaf size and character. The short photoperiodicity gives reciprocal reactions, causing the females to reverse to the male condition and the males to the female condition. Where did the female get her male determiners when the XX formula denied them to her? The only comment necessary is that these false assumptions are apparently perpetuated as true long after they have been discredited. I can grow the hemp year after year with normal, dioecious expression. I can grow the same kind of hemp year after year with mixed sex expression in the individuals. Now what is the normal expression? There is no such thing in heredity unless we mean by normal only the usual to which we have become accustomed. The bisexual expression is just as normal as the unisexual. Before we can insist on normality, we

will have to establish a definite measurable complex of environmental conditions in which the species is supposed to grow and react. This is usually not done. In fact I know of no standard of normal that has ever been adopted for any plant. When we speak of the heredity it is just as proper to insist that the usual hemp is a plant with bisexual individuals six to eight inches tall as to insist that it is a plant with unisexual individuals ten to twelve feet tall.

Just the fact that there apparently is a correspondence of an AA set of allosomes with carpellateness and of an AB set of allosomes with staminateness establishes no presumption whatever that these bodies or any potentialities they may contain are sex determiners. Such an assumption has as much scientific basis as the assumption that horse hairs change to thread worms in watering troughs. Mere correspondence of phenomena does not establish causal relations, and we know that when we cause sex reversal in an allosome-bearing organism there is no correspondence, just as there is no general correspondence between the sex of the gametophytes of the higher plants and the allosomes which they may possess. Even the assumption that an allosome correspondence with sex is always established in the diploid egg has not been extensively investigated, at least in the higher plants.

FROM ANNUALS TO "QUADRENNIALS"

When hemp is grown in the usual spring and summer environment it is strictly an annual. The staminate plants after blooming die promptly when all the conditions of moisture, temperature, light and substratum are still entirely satisfactory for growth. The carpellate plants live longer but also die when the seed is matured. When the plants are grown in the greenhouse and are through blooming and beginning to go into senile decline, if they are then suddenly transferred into continuous light for a certain period of days they will promptly re-

juvenate, as indicated above, pass through a second ontogenetic cycle and bloom and fruit a second time. They will rejuvenate at least twice more under the same kind of treatment, thus passing through four ontogenetic cycles and living over four times as long as their usual life period. If we ask what the specific cause or determiner of this fourfold ontogenetic cycle might be, it is evident that it is the peculiar environment in which the plant is developed. The ability to grow four times and reproduce four times is just as much the "hereditary" nature of the hemp as the ability to reproduce once and then die. But whether it is to die at the end of the first reproductive cycle, at the second, at the third or at the fourth is entirely determined by the environmental factors of the protoplast. All the protoplast determines in the matter is that it is a mechanism that will react in these ways, whenever the proper environmental determiner is present. The real hereditary nature can be defined only in terms of the interrelation of the mechanism of the protoplast and the environment. In fact, the hereditary nature can be discovered only after the organism has been subjected to all possible environmental conditions with survival value.

Development of Peculiar Leaves During Rejuvenation

In the hemp the physiological state or the development of the functional gradient determines the extent of the compounding of the leaf. The cotyledons are simple, entire blades. Beginning with the first leaf node the leaves pass from the simple type gradually to digitate leaves with about fifteen leaflets on very large stems and then down to the simple condition again in the bracts of the inflorescence. The compounding of the leaves, then, depends on the functional state of the protoplasts involved. One can even grow small plants with no compound leaves whatever. Now when the plant is rejuvenated from a lateral bud or from the very tip of the in-

florescence, it is evident that a retrograde functional gradient is at first established which passes from the senile condition to the juvenile condition. Usually, then, the first leaf will be compound again with three leaflets because although partly rejuvenated it is not yet in a proper condition to express simple leaves, but these soon follow and after several nodes have developed the compounding appears again and proceeds through a progressive and regressive complexity in the same way as in the first ontogenetic cycle. Usually the first leaves in the rejuvenation zone are entire, just like the cotyledons, and after a number of such leaves have been developed they again show the usual serration. Now under the usual expression the hemp does not produce such entire leaf and leaflet blades. It is an entirely new kind of leaf just as specific in appearance as if a definite hereditary potentiality for entire leaf character had for the time being been added. Not only do the leaves on the rejuvenation zone develop entire margins, but if there is a return from an extremely senile condition they commonly develop revolute margins and may be otherwise distorted, crinkled or coiled into unsymmetrical, one-sided structures. Occasionally one side of the leaf fails to develop while the other develops normally. Such a one-sided expression makes the leaf appear as though one side of a normal blade had been cut off. Now this new unsymmetrical or unilateral development of a bilateral organ is not caused by the addition or subtraction of "genes" but simply by a disturbance in the physiological reaction of the system which produces a change in the correlation reaction of the growing and expanding tissues. Again the hemp plant in its usual life shows no such characters. There are many species of plants which regularly develop revolute leaves, but they are not in evidence in the usual hemp. Some one might say there was a latent "revolute gene" which just comes to activity in the special physiological state developed in the rejuvenation zone. But I see no reason whatever for such

an assumption. We do not need to change mechanisms to get diversity of activity.

Flowers on the Petioles

Hemp may be so developed in a given short-light-temperature complex that some of the petioles at the base of the inflorescence will bear one or more flowers on the upper side. Sometimes there is a regular row of flowers on the petiole. Now is it the hereditary nature of the hemp to produce flowers on the leaf petiole? I would say that it most certainly is. But again this reaction is determined by a certain specific aggregate of ecological conditions. Under one set of ecological factors the hemp has such a reaction; under another set of ecological factors no such reaction takes place.

A Definition of Heredity

In dealing with the facts and phenomena of heredity in plants and animals, at least four fundamental conditions enter into the problem and determine the nature of the character produced in any given time and place. First, we have the mechanism of the protoplast or group of protoplasts. The protoplast is made up of a system of self-perpetuating protoplasmic units, or autogens. These units not only build up their own structures but divide and segregate in such a way that daughter protoplasts are produced having the same structures and properties. Thus types of plants and animals may continue with unchanged protoplast mechanisms for millions of years. Second, when the sex potentiality is present, as in all higher plants and animals, there is a regular aggregation and segregation of the parts of the protoplasts, the aggregation taking place in fertilization and the segregation in the reduction phase. Since the nucleus is organized from definite individual chromosomes, which are shifted in a definite way during fertilization and reduction, and since the chromosomes contain important hereditary potentialities, these potentialities will be distributed

and combined in definite proportions, thus giving rise to Mendelian segregations and combinations. Since one mass of protoplasm in the protoplast may react on another we have the phenomena of dominance, recessiveness and intermediate reaction; and since the self-perpetuating chromosomes and chromosome particles are subject to abnormal mechanical disturbance and accidents we have the phenomenon of disturbed Mendelian ratios, partly grouped under the common term "crossing-over." Third, since living protoplasts grow, divide and pass through definite ontogenetic cycles, physiological gradients develop which determine the course of differentiation in the organism. Fourth, since the organism is a material thing it can exist and function only in an environment of some kind. The characters of an organism are built up through the physiological activity of individual cells. The physical, chemical and structural activities change with the specific internal and external environmental conditions surrounding the functioning mechanism of the protoplast. Thus the character changes with the environment. The environment has potentiality as well as the protoplast. The immediate environment at the beginning of growth determines the initial activity of the mechanism, and the direction of the movement of the ontogenetic gradient. When this is established it is often difficult to undo what has been done and to start a different reaction series. Nevertheless de-differentiation can be accomplished, and then entirely unexpected developments may take place. For example, a moss spore has the haploid number of chromosomes and is surrounded by a thick cell wall. When it is germinating on moist earth it thus has very definite internal and external conditions, and under these conditions a protonema is developed. On the other hand, the diploid oospore is a thin-walled spore enclosed in the venter of the archegonium, and instead of producing a protonema, on germinating, it grows into a solid aggregate and so continues to the end of its ontogeny. However, we know that it is not the heredi-

tary potentiality or the diploid number of chromosomes that is responsible for this profoundly different result. If a piece of this very diploid tissue is developed in a new environment by planting it in a proper nutrient substratum, it promptly develops a protonema like the haploid spore. The environment determines the specific reaction. There are always two general factors in the equation which determine the given expression—the protoplasmic mechanism and the ecological condition. A change of the proper magnitude in either results in a change of character. Heredity may thus properly be defined as follows: Heredity is the expression or repetition, from generation to generation, or from time to time, of the characteristics of an organism, as determined by a given set of definite, self-perpetuating, protoplasmic units and by a given complex of definite environmental conditions.

The protoplasmic units, or autogens, perpetuate themselves by assimilation. There is no evidence at present that the assimilative process is changed by the presence in the same field of other autogens. Thus a dominant or recessive may be associated or be thrown into all sorts of varietal protoplasmic fields and nevertheless continue their self-perpetuating mechanisms, with their peculiar reaction properties entirely unimpaired. The autogens, whether in the nucleus or cytoplasm, are segregated quantitatively through division during karyokinesis, and so a definite type of protoplast is perpetuated indefinitely. The sex potentiality introduces a new condition resulting in aggregations and segregations of autogens, giving new combinations or new types of protoplasts without the mutation of a single autogen taking place at the time.

At present it would seem unreasonable to assert that the theory of hereditary unit potentialities, whatever they are called or whatever their basis may be assumed to be in the constitution of the protoplast, really explains or takes account of the most important aspect of the problem of heredity and evolution, namely, the self-perpetuating constitution of the organism through which the phylum,

class, order, family and genus peculiarities are continued, practically undisturbed by all the possible hybrid combinations and segregations, by all the extreme fluctuations induced by the environment and by all the multitudinous mutations which have brought about the species in a genus, the genera in a family, the families in an order, etc. These characteristics when once established remain essentially unchanged through the geological ages. Just as the changes brought about by environmental conditions do not really disturb the important qualities, do not change the assimilative nature of the protoplasmic autogens, so all the changes brought about by hybridization and by recognized mutations of new units, after all, do not disturb the great, distinguishing characters of the organism. In both these experimental methods we are not really getting at the fundamental nature of the organism or the protoplast as a whole. We are not disturbing the phylum, class, order, family and genus characteristics, which we assume have evolved by successive changes from common ancestral types. In the taxonomic system, when a family complex is established, the new genera evolved in the group do not lose the family characters and the new species evolved in the genus do not obliterate the generic characters. So with all the multitude of mutations, hybridizations and segregations that have been observed in Oenothera, we still have an Oenothera which any amateur would recognize as an Oenothera at a glance. We do not get rid of "Oenotheraness." The same is true of Drosophila with its multitude of mutations, hybridizations and segregations. After all is said and done, we still have the little fruit-fly. We have not been dealing with or got rid of the "Drosophilaness." At present, the units which we bandy about in our genetic experiments and discussions are no more potent in bringing about a taxonomic system than are the fluctuations which we induce in the individual through diversity of ecological conditions.

LITERATURE BEARING ON THE PROBLEMS DISCUSSED

Garner, W. W., and Allard, H. A.
1920. "Effect of the Relative Length of Day and Night and Other Factors of the Environment on Growth and Reproduction in Plants," *Jour. Agri. Res.*, 18: 553–606.
1925. "Localization of the Response in Plants to Relative Length of Day and Night," *Jour. Agri. Res.*, 31: 555–566.

McClelland, T. B.
1928. "Studies of the Photoperiodism of Some Economic Plants," *Jour. Agri. Res.*, 37: 603–628.

Owen, F. V.
1927. "Hereditary and Environmental Factors that Produce Mottling in Soy Beans," *Jour. Agri. Res.*, 34: 559–588.

Riddle, Oscar.
1928. "The Control of Heredity," *Proceedings* of the Third Race Betterment Conference, Jan. 2–6, pp. 62–71.

Schaffner, John H.
1919. "Complete Reversal of Sex in Hemp," *Science*, 50: 311–312.
1921. "Influence of Environment on Sexual Expression in Hemp," *Bot. Gaz.*, 71: 197–219.
1923. "Sex Reversal in the Japanese Hop," *Bull. Torr. Bot. Club*, 50: 73–79.
1923. "The Influence of Relative Length of Daylight on the Reversal of Sex in Hemp," *Ecology*, 4: 323–334.
1925. "The Influence of the Substratum on the Percentage of Sex Reversal in Winter-grown Hemp," *Ohio Jour. Sci.*, 25: 172–176.
1926. "The Change of Opposite to Alternate Phyllotoxy and Repeated Rejuvenations in Hemp by Means of Changed Photoperiodicity," *Ecology*, 7: 315–325.
1928. "Further Experiments in Repeated Rejuvenations in Hemp and their Bearing on the General Problem of Sex," *Amer. Jour. Bot.*, 15: 77–85.

Thompson, H. C.
1929. "Premature Seeding of Celery," *Cornell Univ. Agri. Exp. Sta. Bull.*, 480: 1–50.

SHORTER ARTICLES AND DISCUSSION

A POSSIBLE ALTERNATIVE TO THE HYPOTHESIS OF SELECTIVE FERTILIZATION IN SCIARA[1]

In an earlier paper in this journal[2] evidence was presented which, at its face value, would indicate that a process of almost completely selective fertilization occurs regularly in the races and species of *Sciara* giving "unisexual" progenies. As noted in that paper, however, "the numerous genetic and cytological peculiarities exhibited by *Sciara* make it desirable to use especial caution in drawing conclusions here." The present paper deals with a possible alternative explanation suggested by further observations on chromosome behavior during spermatogenesis. This alternative is not proposed as a more probable explanation, but merely to indicate the need of reserving judgment even when the evidence for selective fertilization appears to be conclusive.

The evidence indicative of selective fertilization may be recapitulated as follows: (1) Progenies from single females are regularly "unisexual," either males or females. (2) Sex of progeny is determined by the female, not the male. (3) The inheritance of sex-linked characters is of the same type as that regularly found in animals where the female is XX and the male XY in sex chromosome constitution. (4) Single males give both sons and daughters in large numbers, when mated to several females, indicating that they produce and transmit both X-bearing and Y-bearing sperms. (5) Evidence seems to be conclusive that no differential mortality occurs which could account for the "unisexual" progenies. These features are treated in detail in the paper cited above. In each case the data appear to be complete. The only questionable element, therefore, would seem to be that of interpretation, and this in turn would seem to be confined to item three, in which the inheritance of sex-linked characters is used as a criterion of sex chromosome constitution and sex chromosome behavior. The mode of inheritance appears to show that the male is XY in constitution and that at

[1] This investigation has been aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

[2] C. W. Metz, "Evidence that 'Unisexual' Progenies in Sciara are due to Selective Elimination of Gametes (Sperms)," American Naturalist, 63: 214–228, 1929.

maturation X and Y segregate at random and are distributed in such a way that half the sperms receive one and half the other. If this does not occur, then evidently some other mechanism or process is involved which gives the same end result.

It may be recalled that among the peculiarities of chromosome behavior during spermatogenesis[3] is the precocious passage of one chromosome to one pole at the second division and the retention of both its daughter halves in the single spermatid resulting from the division. As a result, all the sperms appear to receive two identical chromosomes of this kind. A recent study of this "precocious" chromosome and its behavior shows that not only does it have spindle fibers extending toward both poles, as previously noted, but that in *S. coprophila,* at least, a small portion of the chromosome appears to be almost, if not completely, detached by the "pull" toward the pole opposite that at which the chromosome lies.

Since the spindle fibers extending in opposite directions are attached to the two respective halves of the split chromosome, the portion just mentioned is a part of only one of these halves. The behavior suggests the possibility, therefore, that from this half a loss of chromatin occurs, and hence that the two sister chromosomes are different when transmitted by the sperm. It is not known whether the "precocious" chromosome is a sex chromosome or an autosome, but if it should prove to be a sex chromosome, this behavior suggests that the alteration might regularly involve the transformation of an X into a Y. In that case the sperms might carry X and Y instead of X or Y. Such a transformation could be brought about either as just suggested, or possibly by a spontaneous chromatin elimination at a later stage.

Any interpretation of this kind would involve numerous difficulties, which need not be reviewed in detail here. On the other hand, however, it would eliminate two of the most serious complications presented by the facts as at present interpreted. These are, first, the selective fertilization already mentioned, and second, the random segregation of X and Y at the monocentric first spermatocyte division,[3] where the autosomes appear to segregate selectively in such a way that in each case only the

[3] Charles W. Metz, M. S. Moses and Ella N. Hoppe, "Chromosome Behavior in Sciara (Diptera). I: Chromosome Behavior in the Spermatocyte Divisions," *Zeit. f. induk. Abstammungs- u. Vererb.,* Bd. 42, Heft 4, 237–270, 1926.

maternal members are transmitted to the resulting single second spermatocyte.

On the scheme just suggested the sex chromosomes would segregate selectively, like the others, in the primary spermatocyte, and the maternal *X* would regularly pass to the second spermatocyte. At the second division it would go precociously to the pole which is to remain in the spermatid, would divide and would have one resulting chromatid transformed into a *Y* chromosome. Each sperm would then carry an *X* and a *Y*, and the selective action exercised by the female would be the elimination of *X* or *Y* at or after fertilization of the egg. The latter process would not present new difficulties in the present case because, as previously noted (*loc. cit.*), we already have to assume a regular elimination of one of these chromosomes (or its homologue in the egg) and likewise we have to assume a selective elimination of the "male-limited chromosomes" in the case of eggs which are to produce females.

A special effort is being made to determine whether or not the "precocious" chromosome is actually a sex chromosome. If it is not, the above hypothesis is automatically eliminated. If it proves to be a sex chromosome, however, then the assumption of selective fertilization must remain questionable, at least until the sex chromosome behavior is fully understood.

Were it not for the apparent absence of synapsis during spermatogenesis in *Sciara*, the above scheme could readily be simplified by the assumption that the "precocious" chromosome in the second spermatocyte was already composed of *X* and *Y* components, because of an equational division of the *XY* tetrad in the first spermatocyte. The cytological evidence, however, is all opposed to this view.

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CHROMOSOME NUMBERS IN CAPSICUM

THE lowest chromosome number yet reported in the Solanaceae, according to Tischler's (1927) list, occurs in *Capsicum annuum* in which Kostow (1926) gave the number in four varieties as n = 6. Margadant (1926), however, gave the count n = 12 for an unstated variety of this species. It is a species which has many advantages for genetical study (see Ikeno, 1913 and 1917,

and Webber, 1911), and a considerable amount of genetic work as yet unpublished was done on it by Mr. G. O. Sherrard when he was at this institution some years ago. If the low chromosome number were confirmed, it would be a species warranting much further study, especially since it will form new shoots from callus tissue on cut-back stems, and might therefore be induced to form tetraploids, as some Solanum species do.

A number of varieties of *C. annuum* were therefore obtained, and chromosome counts made of them. The counts were made from pollen mother-cells mounted in acetocarmine. In addition, counts were made of *Solanum capsicastrum* and of two varieties of *Capsicum baccatum*. In each case the chromosome number was found to be n = 12. The following were the varieties examined:

C. annuum "Long Red"	Plants kindly supplied by Messrs. Sutton and Sons, Reading
" " "Large Red"	
" " "Long Yellow"	
" " "Large Yellow"	
C. baccatum "Long Red"	
" " "Long Yellow"	
C. annuum "Piment gros long changeant"	Seed from Messrs. Vilmorin et Cie.
" " "Piment jaune demi-long d'Antibes"	
" " "Piment jaune long"	
" " "Piment cerise"	

Solanum capsicastrum Large-berried variety; unknown origin

No special peculiarities were observed in the chromosome behavior, and all appeared to be normal diploid species. There appeared to be no features warranting particular discussion or illustration.

These counts were communicated to Professor Tischler, who kindly sent in response a copy of Kostow's paper, which we had not previously been able to obtain. We find from this that Kostow states the chromosome number to be 12 at metaphase and that his photomicrographs show clearly this number of *bivalents*. On the other hand, his figures showing six chromosomes being distributed to either pole are not convincing. We conclude that n = 12 is the correct chromosome number for *Capsicum annuum*.

C. L. Huskins
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LITERATURE CITED

Ikeno, S.
1913. "Studien über die Bastarde von Paprika (*Capsicum annuum*)," *Z. indukt. Abstamm.- u. Vererbungslehre,* 10: 99–114.
1917. "Studies on the Hybrids of *Capsicum annuum,* Part II," *J. Genet.,* 6: 201–229.

Kostow, D.
1926. "Die Bildung der Pollenkörner bei einigen Varietäten von *Capsicum annuum,*" *Jahrb. d. Univ. Sofia,* 4: 101–126.

Margadant
1926. Verbal communication cited by Tischler.

Tischler, G.
1927. "Pflanzliche Chromosomenzahlen," *Tabulae Biologicae,* 4: 1–83.

Webber, H. J.
1911. "Preliminary Notes on Pepper Hybrids," *Am. Breeders' Assoc. Rep't,* 8: 188–199.